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## Perspective

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### Cyclin-Dependent Kinase Inhibitors: Useful Targets in Cell Cycle Regulation

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#### Introduction

Cancer drug discovery is one of the most rapidly changing areas of pharmaceutical research. Most anticancer agents that are approved for clinical use are molecules which damage deoxyribonucleic acid (DNA), block DNA synthesis indirectly through inhibition of nucleic acid precursor biosynthesis, or disrupt hormonal stimulation of cell growth.<sup>1</sup> Many of these agents are widely used and have provided therapeutic benefit to many. However, attempts to modify these agents to improve efficacy (i.e. clinical response rate) or scope of activity with respect to tumor type have been largely disappointing. In the 1990s, two classes of natural product-derived anticancer compounds, the taxanes and the camptothecins, have provided new clinical agents with significantly improved levels of efficacy.<sup>2,3</sup> The basis for their somewhat selective action against tumors remains, as it does for the more traditional agents, poorly understood.

Although efforts to refine and incrementally improve existing classes of anticancer agents continue, there has been a recent shift of emphasis toward novel mechanistic targets that have emerged as a direct consequence of the intense study of the underlying genetic changes associated with the cancerous state. The identification of tumor suppressor genes that are lost or inactivated and oncogenes that are activated in tumors has suggested many pathways and specific molecular entities as rational targets for anticancer drug discovery. For example, the early identification of the *ras* oncogene and its clinical significance stimulated interest in the targeting of farnesyl transferase as a means of controlling p21<sup>ras</sup>-driven pathways.<sup>4</sup> Several farnesyl transferase inhibitors are being clinically evaluated at this time.

More recently, pharmaceutical attention has been focused on the proteins that drive and control cell cycle progression. The significance of these potential drug targets is clearly evidenced by the high incidence of alterations in the genes that code for these proteins in tumors. A key class of cell cycle proteins is the cyclin-dependent kinases (CDKs). The CDKs activate host proteins through phosphorylation on serine or threonine using adenosine triphosphate (ATP) as a phosphate donor.

Targeting of protein kinases is a relatively recent activity for medicinal chemists. A number of natural product-derived kinase inhibitors have been discovered and characterized.<sup>5,6</sup> The ATP-competitive nature and the broad activity of many of these agents against a range of protein kinases led many to believe that it would be difficult, if not impossible, to selectively target a single kinase. This belief was challenged in 1994 in a publication describing an exceptionally potent inhibitor of epidermal growth factor receptor tyrosine kinase that was ATP-competitive and highly specific relative to closely related receptor tyrosine kinases.<sup>7</sup> Since that time, a number of laboratories have reported similarly on highly potent and specific inhibitors for other receptor tyrosine kinases.<sup>8</sup> While the clinical utility of this class of agents and the generality of these findings remain to be determined, it is clear that at least some protein kinases appear to be medicinally "targetable" with compounds having useful potency and specificity.

The medicinal literature on kinase inhibition is very much in its infancy with trends and design principles just now emerging. In this Perspective, we will review the status of efforts directed specifically toward the discovery of potent and specific inhibitors of cyclin-

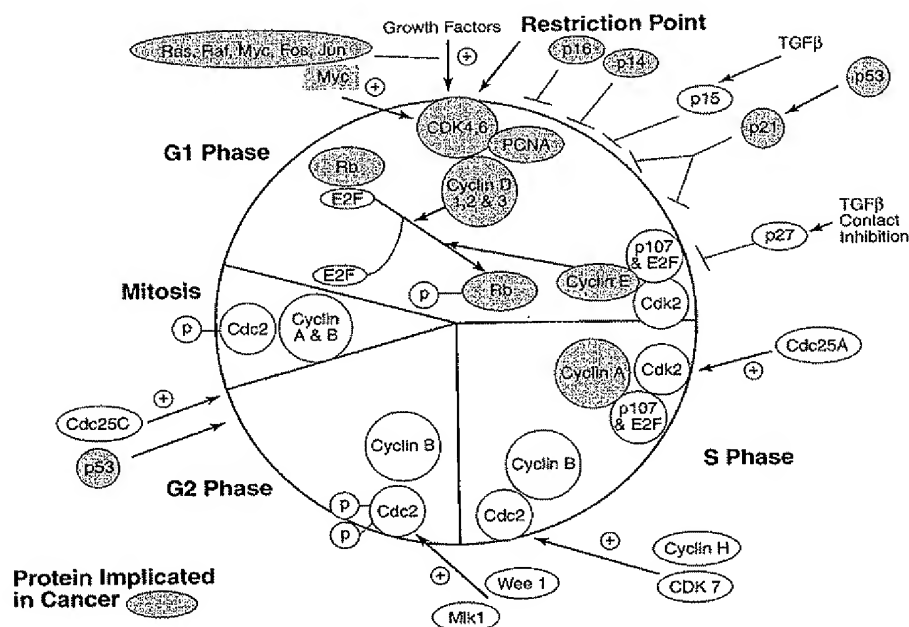


Figure 1. Cell cycle regulatory components determined to be altered in human cancers.<sup>12</sup>

dependent kinases (CDKs). Such inhibitors are expected to be useful in the treatment of proliferative disorders, including cancer, and it is hoped that the design principles gleaned from these efforts will facilitate the targeting of a wide range of protein kinases with broad therapeutic potential.

### The Cell Cycle

CDKs have attracted attention as potential therapeutic targets because they are key players in the control of cellular proliferation.<sup>9–11</sup> Eukaryotic cell division is driven by a regulated series of events collectively defined as the cell cycle. The principal function of the cell cycle is to effect the duplication of DNA and its appropriate distribution to newly divided daughter cells. Although strictly a continuum of events, for convenience the cycle has been described as falling into four phases. DNA replication occurs in S phase, and replicated DNA is distributed to progeny cells in M phase. Between these two phases are periods originally called gaps: gap 1 (G1) between M phase and S phase and gap 2 (G2) between S phase and M phase (see Figure 1). Thus G1 cells have a normal diploid (2N) DNA content, whereas G2 cells have a 4N DNA content. S phase cells are actively duplicating DNA and thus have a DNA content between 2N and 4N. Lengths of the individual phases of the cell cycle can vary with cell type and with conditions. In some rapidly dividing cells the G1 and G2 phases can be very short or absent. In addition, cells can reversibly exit the cell cycle at the end of M phase and enter a state known as G<sub>0</sub>, or they can irreversibly exit the cycle as happens during senescence or terminal differentiation.<sup>12</sup> (See Table 1 for terminology.)

**The CDKs.** Progression through the individual phases of the cell cycle is driven by CDKs.<sup>13</sup> This family of kinases requires association with a cyclin regulatory

subunit for activity. Different cyclin/CDK pairs are active during each phase of the cell cycle (see Figure 1).<sup>12</sup> To date at least nine CDKs and more than twelve different cyclin families have been described. Individual family members have a limited ability to assort as heterodimeric complexes. Critical cyclin/CDKs for core cell cycle function are cyclin D/CDK4(6), cyclin E/CDK2, cyclin A/CDK2, and cyclin B/CDK1 (also known as cdc2). The D type cyclins D1, D2, and D3 associate with CDKs 4 and 6 and are believed to play an important role early in G1. The D type cyclin/CDKs collaborate with cyclin E/CDK2 to drive cells into S phase and through a point in the cell cycle called the restriction point.<sup>14</sup> The restriction point represents a key stage in the life cycle of a cell in that once cells have traversed this point they become committed to cell cycle entry and no longer need growth factor input for further cell cycle progression. Further progression into S phase is driven by cyclin E/CDK2, and thereafter cyclin A/CDK2 promotes the further completion of S phase. CDK1 in association with cyclins A and B completes the cycle by controlling G2 progression and subsequent DNA segregation and then separation to two daughter cells during M phase and subsequent cytokinesis.

In addition to playing a key central role in cell cycle control, CDKs have also been described as potentially serving additional functions.<sup>15</sup> Some members of the CDK family play a regulatory role in controlling the activity of cell cycle CDKs. These are collectively termed the CDK-activating kinases or CAKs.<sup>16</sup> The role of these kinases is to control the activity of cell cycle CDKs by phosphorylation of critical serine and threonine residues needed for activity (see below). Some of these CAKs appear to have a dual role in that they are also able to phosphorylate the C-terminal domain of RNA polymerase II and control transcription.<sup>17</sup> Other CDKs have been described with an as yet uncertain function. One of these, CDK5, has an atypical regulatory subunit (p35)

**Table 1.** Terminology Review

cell cycle function	protein family	specific members	other names	equivalents in yeast
cyclin/CDKs	G1 cyclin/CDKs	cyclins D1–D3/ CDK4,6		CLN1,2,3/CDC28: budding yeast
		cyclin E1,E2/ CDK2		CIG1/CDC2: fission yeast
	S phase cyclin/CDKs	cyclin A/CDK2		CLN5,6(3,4)/CDC28: budding yeast CIG2/CDC2: fission yeast
	G2 phase cyclin/CDKs	cyclin B/CDC2	cyclin B/CDK1	CLB1,2(3,4)/CDC28: budding yeast CDC13/CDC2: fission yeast
downstream CDK substrates	RB (tumor suppressor) principal substrate for CDK4 RB and many others for CDK2	RB, p107, p130		none
CDK modifying enzymes	CAK (CDK activating enzyme)		cyclin H/CDK7	CAK
	hwee1 (CDK inhibitory kinase) phosphorylates Tyr-15 on CDC2			wee1: fission yeast swe1: budding yeast
	hmyt1 (CDK inhibitory kinase) phosphorylates Thr-14 on CDC2			myt1: fission yeast
	CDC25 (phosphatase that removes inhibitory phosphorylation on Thr-14, Tyr-15)	CDC25 A, B, C		mik1: fission yeast CDC25
CDK inhibitory proteins	p16	p16	INK4a, MTS1, CDKN2	none
		p15	INK4b, MTS2	
		p18	INK4c	
		p19	INK4d	
	p21	p21 p27	Cip1/Waf1 Kip1	p25rum1: fission yeast p40sic1: budding yeast
		p57		
CDK assembly factors	hcdc37			cdc37
RB interacting proteins	MAT1			
	E2F	E2F 1–5, DP1–3		none

distinct from other members of the cyclin family.<sup>18,19</sup> CDK5 activity is highest in the brain.

**Cell Cycle Progression – CDK Substrates.** *G1 Phase:* Transit through the cell cycle is the result of sequentially controlled waves of cyclin/CDK activation. Less clear is the nature of the critical substrates for each of the activated kinases. Entry into the cell cycle at G1 and passage through the restriction point are controlled by the combined activity of cyclin D/CDK4 and cyclin E/CDK2.<sup>20,21</sup> A key substrate and the only extensively documented substrate of cyclin D/CDK4 is the product of the tumor suppressor gene product retinoblastoma.<sup>22</sup> The retinoblastoma protein contains multiple potential CDK phosphorylation sites, and different CDKs have been reported to have different site preferences for phosphorylation of this substrate.<sup>23</sup> Members of the retinoblastoma gene family are transcriptional corepressors.<sup>24</sup> Key among retinoblastoma interacting proteins are members of the E2F family of transcription factors. Phosphorylation of Rb leads to the release of bound E2F and activation of downstream E2F genes which include a number of genes important for DNA synthesis and subsequent cell cycle progression, e.g. tk, dhfr, cyclin E, and the retinoblastoma protein itself.<sup>25–27</sup> Thus at least part of the role of the G1 cyclin/CDKs is to control transcription of genes important for subsequent cell cycle progression. In addition, it seems likely that cyclin E/CDK2 has additional substrates besides Rb that likely include proteins involved in assembly of the replication machinery.<sup>28,29</sup> Phosphorylation of Rb appears to correspond to transition through the restriction point.<sup>30</sup> The critical role for cyclin D/CDK4 in this process is underscored by the fact that the natural

specific inhibitor of cyclin D/CDK4, p16, is inactive in cells that are deficient in Rb function.<sup>31</sup> However, Rb-negative cells are capable of exhibiting a controlled cell cycle, and it has been shown recently that Rb-positive mouse fibroblasts can cycle in the almost complete absence of cyclin D/CDK4,6 activity.<sup>32</sup> In addition mice in which CDK4 activity has been deleted develop normally with minor abnormalities in the pancreas.<sup>33</sup> Thus the precise role of cyclin D/CDK4,6 in core cell cycle control is still somewhat open to speculation.

G1 progression represents a phase in the cell cycle which is highly responsive to growth factor stimulation.<sup>34</sup> Growth factor signaling impacts cyclin D synthesis and stability, cyclin D/CDK4,6 complex assembly, and CDK activation.

*S Phase:* Once cells have passed the restriction point and committed to S phase, they become independent of growth factor stimulation for continued cell cycle progression. The precise role that CDKs play in control of DNA replication is not defined. Both cyclin E/CDK2 and cyclin A/CDK2 complexes appear to play an important role during S phase. Cyclin E/CDK2 appears to play a critical role in centrosome duplication,<sup>35</sup> and CDK2 is necessary for DNA replication in a cell-free system.<sup>36</sup> The critical substrates for cyclin E/CDK2 and cyclin A/CDK2 have not been defined.

*G2/M Phase Regulation:* Following completion of DNA replication, cells enter the G2 phase of the cycle with a 4N DNA content and begin preparations for M phase and cytokinesis. CDK1 has been shown to regulate passage of cells through these later phases of the cell cycle in association with both cyclins A and B. Complete activation of CDK1 requires both cyclin bind-

ing and phosphorylation of Thr-160/161.<sup>37</sup> CDK1 activity can be negatively regulated by phosphorylation on Thr-14 and Tyr-15.<sup>38</sup> This negative control pathway provides a point of intervention of checkpoint control pathways that halt the cell cycle if DNA replication is incomplete, DNA is damaged, or spindle assembly has not occurred correctly. Once activated CDK1/cyclin complexes prepare the cell for division during M phase. A range of potential substrates includes proteins that regulate chromosomal condensation, formation of mitotic spindles, and fragmentation of the Golgi apparatus.<sup>39</sup>

**CDKs and Cell Cycle Regulation.** Cell cycle regulation is complex reflecting the importance of this key biological process. To maintain the correct cellular DNA content it is critical that mechanisms are in place to control the correct timing of entry into the cycle and to allow an orderly progression of events to avoid damage to the genetic material. Activities needed for passage through any given phase of the cycle need to be activated at the correct time and then inactivated once that phase is complete. For example, it is important that cell division does not occur if DNA is damaged or if replication and spindle assembly are not complete. For multicellular organisms it is also important to allow for appropriate cell cycle exit during terminal differentiation and senescence.

Consistent with this the activity of CDKs is tightly controlled during the cell cycle. Individual complexes are sequentially activated and deactivated. Multiple mechanisms exist to enable this to occur, and CDK activity is controlled at many levels.<sup>40</sup> These include (a) association with activating cyclin subunits, (b) synthesis and degradation of these cyclin subunits, (c) posttranslational modifications driven by kinases and phosphatases, and (d) interactions with natural inhibitors/activators.

**Cyclin Association:** As indicated earlier CDKs require association with the appropriate cyclin subunit for activity. This association results in structural changes in a region of the CDK called the T-loop allowing access of the active site to substrate.<sup>41</sup> In addition it has been proposed that the cyclins play an important role in determining cyclin/CDK substrate specificity, and important substrate binding sites may reside on this subunit.<sup>42</sup>

**Protein Synthesis and Degradation:** Although CDK levels remain fairly constant in proliferating cells, cyclin levels oscillate through the cell cycle with the levels of individual cyclins peaking at different times. Increases in cyclin levels are driven by increases in rates of synthesis. Thus synthesis of cyclin D in early G1 phase is under growth factor control, and the cyclin D promoter contains several regulatory elements sensitive to growth factor control.<sup>43</sup> Conversely ubiquitin-mediated proteolysis appears to play a key role in controlling cyclin down-regulation.<sup>44</sup> The process of cyclin/CDK assembly is also controlled although this process is less clearly understood. Several proteins have been proposed to play a role in cyclin D/CDK4 assembly, e.g. MAT1<sup>45</sup> and cdc37.<sup>46</sup> In addition it has been reported that the p21 family of low molecular weight CDK inhibitors might also provide an assembly role for cyclin D/CDK4 while being inhibitory for other cyclin/CDK pairs thus

adding an additional level of complexity and sophistication to the cell cycle engine.<sup>47</sup>

**Posttranslational Modification:** Phosphorylation represents the most critical cyclin/CDK modification event described to date. Both activating and inactivating phosphorylations control CDK activity, and cyclin degradation is subject to control by phosphorylation. Typically, CAKs control activating phosphorylations on CDKs that result in correct structural orientation of residues surrounding the active site, e.g. Thr-160/161 in CDK1.<sup>48</sup> Additional kinases are able to catalyze phosphorylation events that result in CDK inactivation. For example, the *wee*/*myt*1 family kinases modify CDK1 on Thr-14 and Tyr-15 resulting in inactivation.<sup>49,50</sup> These inactivating phosphorylations can be reversed by the *cdc25* family of protein phosphatases of which three members (*cdc25A*, *B*, and *C*) have been described that are active in different phases of the cell cycle.<sup>51,52</sup>

**Low Molecular Weight Protein Inhibitors/Activators:** Finally, cells express at least two families of low molecular weight proteins that serve as natural inhibitors of cyclin/CDKs: the p21 gene family (p21, p27, p57) and the p16 gene family (p15, p16, p18, p19).<sup>53</sup> Members of the p21 gene family are able to interact with both the cyclin and CDK subunits. Although originally described for their cyclin/CDK inhibitory role, p21 and p27 have now been found in catalytically active complexes of cyclin D/CDK4, and it has been suggested that they are necessary for efficient assembly of this CDK complex in its active form while serving an inhibitory role for other cyclin/CDKs, e.g. cyclin E/CDK2.<sup>32,47</sup> Members of the p16 gene family interact only with the CDKs and are specific in their interaction with CDK4 and CDK6 where they are able to inhibit the activity of complexes containing these CDKs.<sup>54</sup>

Thus a complex network of posttranslational modification events provides the cell with additional regulatory flexibility with respect to cell cycle control. It is likely, however, that in the cell cyclin/CDK complexes exist in multiple heteromeric complexes with potential substrates and regulators. It is probable that dynamic regulation and assortment of these complex components provide for an additional level of regulatory complexity.<sup>55</sup>

**Checkpoint Control.** One of the benefits to the cell of this complex regulatory machinery is the ability to manifest checkpoint control. Originally defined by Hartwell and colleagues,<sup>56</sup> checkpoint control affords the cell the ability to ensure the orderly progression of cell cycle events. Cells continually monitor both their external and internal environment to ensure that cell cycle progression is appropriate. All phases of the cell cycle are subject to checkpoint control. Important and much studied checkpoints occur at G1 and at G2/mitosis. The G1 checkpoint serves to ensure that cells do not begin DNA synthesis unless they are appropriately nourished, they are correctly interacting with other cells or their substratum, and DNA is intact.<sup>57</sup> The replication<sup>58-61</sup> and mitotic<sup>62,63</sup> checkpoints serve to halt the cell cycle if DNA replication is not complete and if correct assembly of the mitotic spindle has not occurred. The response of the cell to inappropriate conditions is removal from the proliferative compartment. This may involve

either a transient arrest until the situation is corrected or a permanent arrest or death. Factors governing transient versus permanent cell cycle withdrawal are incompletely understood at the present time.

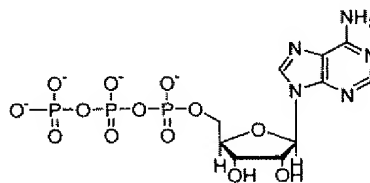
**Cell Cycle Control and Disease.** Cell cycle dysregulation has important consequences in relation to human disease. Inappropriate cell cycle progression is a critical feature of tumor cells, and it is the hallmark of transformed cells that they lack appropriate checkpoint control. In this respect deviations in G1 cell cycle control have been most studied. The Rb kinase pathway has been reported to be heavily mutated in human tumor cells, and it has been estimated that over 90% and potentially all human tumor cells contain mutations in this pathway.<sup>64-67</sup> Mutations and/or deregulated expression in human tumors have been described for Rb, cyclin D, CDK4, cyclin E, and the p16 gene family.<sup>68</sup> In addition p21 is a downstream target of p53, the most commonly mutated gene in human cancers. Figure 1 indicates the cell cycle regulatory components that had been identified as altered in human cancers at the time of writing of the Hunter and Pines review in 1996. It clearly shows a concentration of mutational events associated with the G1/S transition. However, in addition deregulation of other phases of the cell cycle may also play a role in human tumorigenesis. Alterations in cell cycle control may not only be relevant to cancer progression. Inappropriate cell cycle entry has been described as contributing to certain neurodegenerative diseases,<sup>69,70</sup> and activation of the cell cycle is targeted by several viruses both transforming and nontransforming to place the host cell in a state conducive to viral function.<sup>71</sup>

This observation has raised a level of interest in the CDKs themselves as potential therapeutic targets of promise in diseases where control of inappropriate cellular proliferation would be of therapeutic benefit. This in turn has led to initiation of strategies aimed at discovery of potential specific low molecular weight CDK inhibitors and their evaluation as therapeutic agents.

### Inhibitors of CDKs

The elucidation of the primary, secondary, and tertiary structures (Figure 2)<sup>72</sup> of CDKs has been a priority in the development of first-generation inhibitors. As noted by Kim<sup>73</sup> there are five regions which show marked differences when comparing the structure of CDK2 in the ATP complex<sup>74</sup> to the ternary structure of CDK2 complexed with ATP and cyclin.<sup>75</sup> These differences are reflected in the two domains which are more open in the ternary complex, the conformation of the triphosphate of ATP (1) which is different in the two structures, and the observation that when bound to cyclin A the  $\alpha/\beta$  transition box is modified, the T-loop is completely reoriented, and the cyclin binding helix is in a different location and orientation.<sup>76</sup>

Of even greater importance to the successful development of small molecule kinase inhibitors is the understanding of the primary interactions of endogenous inhibitors to their substrates. p21 is a universal inhibitor of the mammalian CDKs via complexation with cyclins, CDKs, and the proliferating cell nuclear antigen. Crystallization of p21 has resulted in diffractable crystals.<sup>77</sup> However, no crystal structure of p21 complexed to a cyclin has been reported.



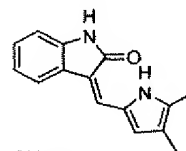
ATP, 1

The structure of p19<sup>INK4d</sup> was also reported based on a series of NMR spectroscopy experiments. It was suggested that the majority of lethal mutations to the p16<sup>INK4a</sup> gene are the result of incorrectly folded and/or insoluble protein.<sup>78</sup> Subsequent publication of the crystal structure for CDK6 bound to p19<sup>INK4d</sup> shows that, as seen in other kinases, CDK6 consists of the catalytic cleft lying between the two domains. p19<sup>INK4d</sup> is made of five ankyrin repeats followed by a helix-loop-helix motif. The consecutive repeats are linked by  $\beta$ -turns between the N- and C-termini. The protein has an elongated L-shape overall. The interface of CDK6 and p19<sup>INK4d</sup> has three main constituents: (1)  $\alpha 1$ ,  $\alpha 2$ , and  $\alpha 5$  helices of p19<sup>INK4d</sup> pack at right angles across the N-terminal  $\beta$ -sheet of CDK6; (2) the  $\beta$ -turns between ankyrins I/II and II/III in p19<sup>INK4d</sup> act as a cap over the CDK6  $\beta$ -sheet; (3) loops L4 and L6 clamp on the C-terminal domain resulting in a twist of CDK6 prohibiting effective ATP binding.<sup>79</sup>

The kinase substrate ATP has been cocrystallized with CDK2 (Figure 3).<sup>73</sup> The key interactions in the ATP pocket include hydrogen bonds between Leu-83 to N1, Glu-81 to N(6)H2, and Asp-86 to O(2')H, in addition to the phosphate chain interactions with the pocket at Lys-33, Asn-132, Asp-145, Lys-129, Thr-14, and Wat-558.

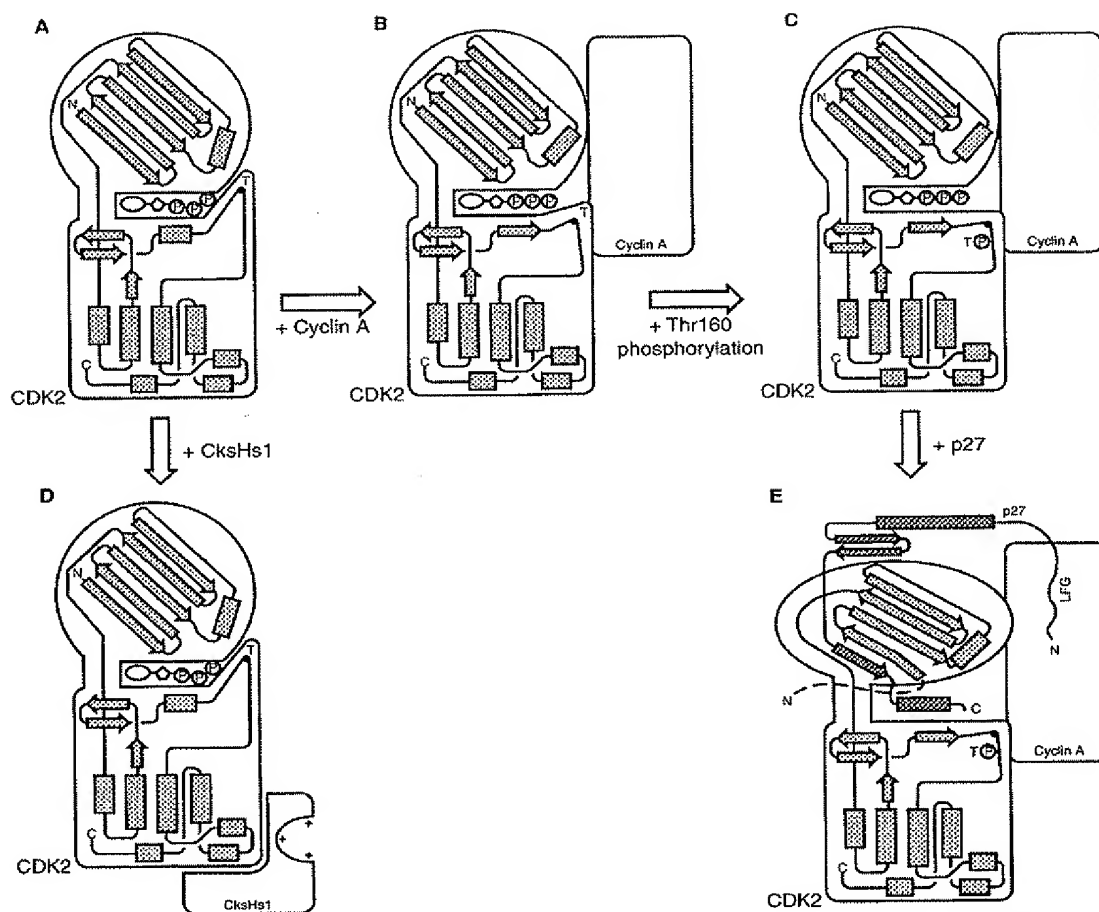
This type of structural information has led to the employment of three main approaches to the inhibition of CDKs which include ATP-competitive inhibitors, noncompetitive inhibitors which bind in the region of the natural peptide inhibitors, and molecules incorporating both of these approaches. The majority of published reports on CDK inhibitors utilize a small molecule approach to develop ATP-competitive inhibitors.

Frequently, first-generation approaches to inhibitors rely on similarities to known inhibitors of analogous substrates. This type of approach has been modestly successful for kinase inhibitors. Indeed, for many kinases, small molecule ATP-competitive inhibitor approaches are being tested in clinical studies. Some of the more advanced tyrosine kinase inhibitors include the 3-substituted indolinone SU 5416 (2) which is in phase II for a cancer indication,<sup>80</sup> while ZD 1839, a substituted anilinoquinazoline is being developed as a treatment for solid tumors.<sup>81</sup> It is interesting to note that both of these kinase inhibitors are relatively flat and contain a high degree of conjugation.



SU 5416, 2

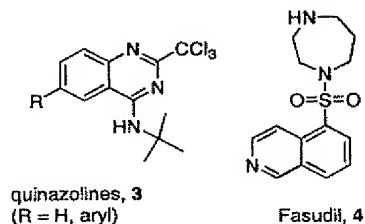
Quinazolines have been used as the core of a number of additional tyrosine kinase inhibitors which are in



**Figure 2.** Schematic representations of the five CDK2 structures determined to date. (A) The inactive CDK2 monomer B, in which the ATP phosphates are misoriented because of the presence of the L12 helix and the protein substrate binding site is blocked by the T-loop (T). (B) The partially active complex of CDK2 and a truncated cyclin A. The ATP phosphates are correctly positioned and the T-loop only partially obscures the active-site cleft. (C) The fully active CDK2/cyclin A complex phosphorylated at Thr-160 in the T-loop. The T-loop lies flat along the base of the active-site cleft. (D) The inactive CDK2 subunit complexed with the CksHs1 protein, which interacts primarily with CDK2 helix 5 and the L14 loop that follows this helix. The positively charged patch on the CksHs1 protein has the potential to interact with phosphates on other proteins and may augment the binding of CDK2 to phosphorylated substrates or regulatory proteins. (E) Phosphorylated CDK2/cyclin A in a complex with the inhibitor p27. The inhibitor interacts extensively with the upper kinase lobe, disrupting its structure and blocking ATP binding. Dashed line represents the disordered amino-terminal region of CDK2. In all panels, dark-gray-shaded rectangles and large dark-gray-shaded arrows represent  $\alpha$ -helices and  $\beta$ -strands, respectively; PST, PSTAIR helix (single-letter code for amino acids), also known as helix 1; N, amino-terminus; C, carboxyl-terminus; circled P, phosphate; T, threonine 160; LFG, p27 motif involved in cyclin binding. Secondary structural elements and loops in this figure and all other figures are not drawn to scale, and interactions between elements have been greatly simplified for clarity<sup>72</sup> (reproduced with permission).

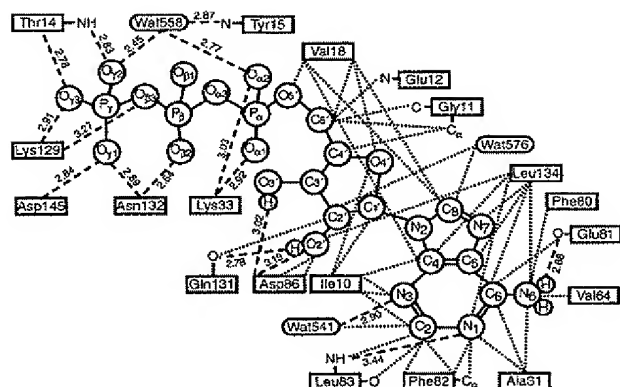
various stages of development.<sup>82–88</sup> Taking advantage of the quinazoline as a strong recognition element for the ATP binding pocket, this core has been utilized as a CDK inhibitor scaffold (3) (Thais M. Sielecki, unpublished results). A variety of alkylamino groups are tolerated at the 4-position, while the 2-position is much more sensitive to alternate substitution. The addition of 6-aryl groups increases the potency of these molecules to the submicromolar region for CDK2 (3, where R = *m*-aminophenyl: CDK2/cyclin E  $IC_{50}$  = 0.65  $\mu$ M, CDK4/cyclin D  $IC_{50}$  = 2.1  $\mu$ M).

PKC inhibitors are also showing utility in clinical studies and include some drugs currently marketed such as fasudil (4), a diazepine developed as a vasodilator which inhibits smooth muscle cell proliferation and DNA synthesis.<sup>89</sup> Staurosporine (5), another ATP-competitive PKC inhibitor, is a natural product isolated



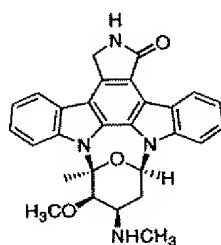
from *Streptomyces staurosporeus*<sup>90</sup> which shows significant CDK inhibition (CDK2/cyclin A  $IC_{50}$  = 7 nM,<sup>91,92</sup> CDK4/cyclin D  $IC_{50}$  = 3–10  $\mu$ M, cAPK  $IC_{50}$  = 8.2 nM).<sup>93</sup> The cocrystallization of staurosporine with CDK2 reveals several key interactions of the inhibitor with the backbone residues lining the ATP binding pocket. Similar interactions are seen in the binding of ATP to CDK2 which include a hydrogen bond to the oxygen of



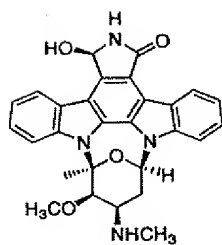


**Figure 3.** All atomic interactions between CDK2 and ATP. Contacts with protein side chains are indicated by lines connecting to the respective residue box, while interactions to main chain atoms are shown as lines to the specific main chain atoms indicated. van der Waals contacts are indicated by dotted lines and hydrogen bonds by thick broken lines. ATP van der Waals contacts to phosphates were omitted for clarity<sup>73</sup> (reproduced with permission).

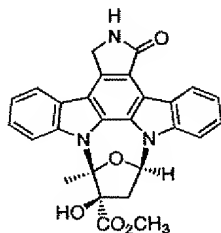
Glu-81 and a hydrogen bond acceptor interaction with the nitrogen of Leu-83. Additional hydrogen bonds are formed between the methylamino group and the carbonyls of Gln-131 and the side chain of Asp-86.<sup>94</sup>



Staurosporine, 5



UCN-01, 6

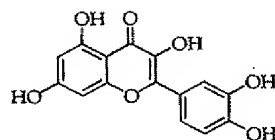


K-252a, 7

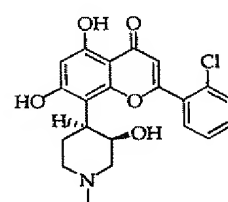
The hydroxystaurosporine analogue UCN-01 (6), isolated from the culture broth of *Streptomyces* sp., is currently in phase I clinical trials to evaluate the compound's tolerability and pharmacokinetics.<sup>95</sup> Additionally, 6 is being evaluated as a radiosensitizer.<sup>96,97</sup> Compound 6 is a nonspecific kinase inhibitor against PKC ( $IC_{50} = 6.8$  nM), CDK1 ( $IC_{50} = 31$  nM), and CDK2 ( $IC_{50} = 30$  nM) while having only weak activity against Raf-1 kinase and MAPK ( $IC_{50} = 620$  and  $910$  nM). In cell cycle analyses 6 inhibits the G1/S transition. Additionally, A549 cells treated with various concentrations of 6 showed a decrease in a dose-dependent

manner in the amount of phosphorylated pRB.<sup>98,99</sup> Compound 6 has also been shown to induce apoptosis in malignant glioma cells<sup>100</sup> and in gastric and breast cancer cells.<sup>101</sup> In response to the challenging chemistry of this series, several syntheses of staurosporine, UCN-01, and the furanyl analogue K-252a (7) have been reported.<sup>102–106</sup> Although this series shows good potency in a variety of kinases, its apparent lack of selectivity presents the possibility for non-mechanism-dependent side effects in a clinical setting.

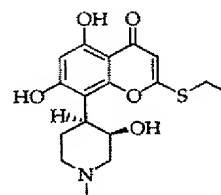
Several flavonoids have proven to be effective at inhibiting CDKs, including quercetin (8), flavopiridol (9), and 2-thioflavopiridol (10). Quercetin is a naturally occurring flavonoid which has shown cardiovascular, anticancer, and analgesic effects. Over a range of concentrations from  $10$  nM to  $10$   $\mu$ M, quercetin has shown a dose-dependent and reversible inhibition of cancer cell proliferation. Cell cycle analysis has shown that cells are blocked at the G0/G1 interface, a result consistent with a CDK inhibitor.<sup>107</sup> Quercetin is a relatively weak CDK inhibitor which does, however, present the flavone core as a viable structural template.



Quercetin, 8

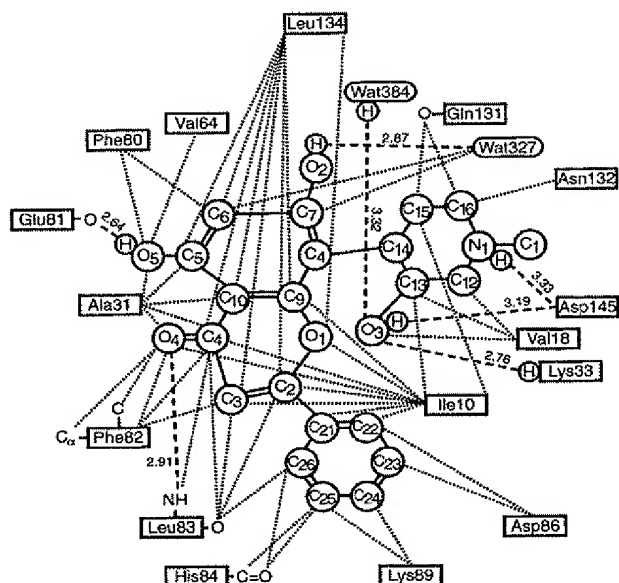


flavopiridol, 9



10

Flavopiridol (9) (NSC-649890, L86-8275) utilizes the flavone core while introducing additional interaction sites through the incorporation of the piperidine at the 8-position and by the modification of the 2-aryl substitution pattern. This flavone is the most advanced CDK inhibitor, currently in phase II clinical trials for refractory myeloma, advanced gastric carcinoma, and high-grade non-Hodgkin's and mantle cell lymphoma.<sup>108–111</sup> The compound is a nonselective kinase inhibitor showing in vitro activity against CDK4, CDK2, CAK (CDK4/cyclin D  $IC_{50} = 0.4$   $\mu$ M, CDK2/cyclin A  $IC_{50} = 0.1$   $\mu$ M, CAK  $IC_{50} = 0.3$   $\mu$ M),<sup>112,113</sup> CDK1 ( $IC_{50} = 0.4$   $\mu$ M),<sup>114</sup> and PKC<sup>115</sup> with very slight activity for EGF-receptor tyrosine kinase ( $IC_{50} = 21$   $\mu$ M) and PKA ( $IC_{50} = 122$   $\mu$ M).<sup>116</sup> When flavopiridol is given daily as an iv bolus in mice, selective apoptosis of cells in the thymus, spleen, and lymph nodes was observed. When dosed at  $7.5$  mg/kg bolus iv or ip in mice on each of 5 days, eleven of twelve advanced stage sc human HL-60 xenografts underwent complete regressions and animals remained disease-free several months after one course of drug. Other lymphomas including SUDHL-4 sc, AS283, and Nalm/6 also showed significant responses to flavopiridol treatment.<sup>117</sup> This flavone has also shown promising



**Figure 4.** All atomic interactions between CDK2 and deschloroflavopiridol. Contacts with protein side chains are indicated by lines connecting to the respective residue box, while interactions to main chain atoms are shown as lines to the specific main chain atoms indicated. van der Waals contacts are indicated by dotted lines and hydrogen bonds by thick broken lines<sup>73</sup> (reproduced with permission).

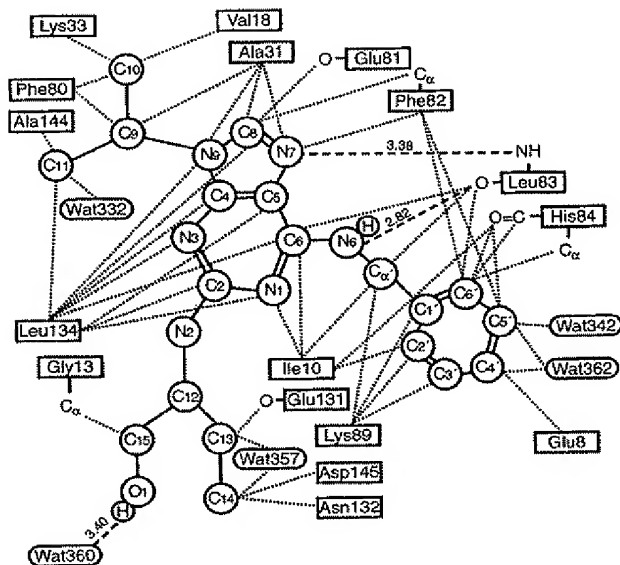
results in combination therapy, enhancing the activity of Taxol in human trials.<sup>118,119</sup>

The X-ray crystal structure of deschloroflavopiridol cocrystallized with CDK2 reveals several key interactions<sup>120,121</sup> (Figure 4).<sup>73</sup> Key hydrogen bonds are formed to Lys-33 and Wat-384 with O(3) of the hydroxypiperidine moiety and to Asp-145 with the N(1)H and O(3)H. Core hydrogen bonds are formed to Glu-81 with O(5)H and to Leu-83 with O(4).

Replacement of the 2-aryl moiety with a thio group (**10**) provides a series that maintains CDK inhibitory activity and has potential utility in the treatment of cancer, inflammation, and arthritis. A representative compound of the 2-thioflavone series is shown; however, no in vitro data has been disclosed in the literature.<sup>122</sup>

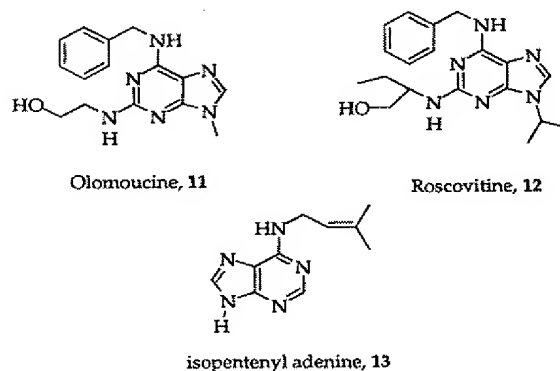
Overall, the flavone core has been one of the more frequently utilized ATP-competitive structural classes. Again, the compounds show a lack of specificity, and this causes concern for possible undesired side effects in the clinical setting.

The purine ring system is used in a number of CDK inhibitors. Included in this category are olomoucine (**11**), roscovitine (**12**), isopentenyladenine (**13**), boheminine (**14**), purvalanol (**15**), and CVT 313 (**16**). Olomoucine (**11**) was identified as an active lead compound through the screening of substituted purines.<sup>123</sup> Showing modest potency for CDK1/cyclin B ( $IC_{50} = 7 \mu M$ ), CDK2/cyclin A ( $IC_{50} = 7 \mu M$ ), CDK2/cyclin E ( $IC_{50} = 7 \mu M$ ), and CDK5/p35 ( $IC_{50} = 3 \mu M$ ), the compound was inactive with respect to CDK4/cyclin D1 and CDK6/cyclin D3 in addition to other non-CDKs including PKC and insulin-dependent tyrosine kinase. A second-generation purine in which the 2- and 9-substituents are increased in size is roscovitine (**12**). This compound shows a 10-fold improved potency for CDK1, CDK2, and CDK5 while



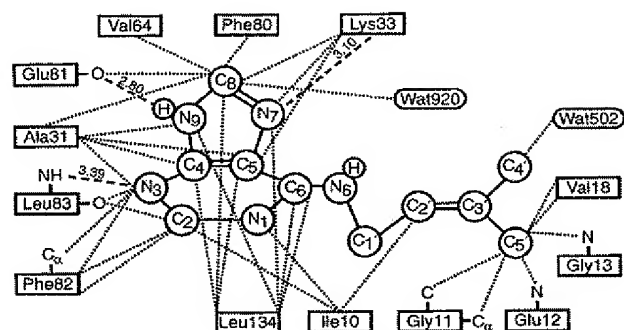
**Figure 5.** All atomic interactions between CDK2 and olomoucine. Contacts with protein side chains are indicated by lines connecting to the respective residue box, while interactions to main chain atoms are shown as lines to the specific main chain atoms indicated. van der Waals contacts are indicated by dotted lines and hydrogen bonds by thick broken lines<sup>73</sup> (reproduced with permission).

maintaining selectivity with respect to a number of other kinases. When tested in cells using a non-small-cell lung cancer cell line, MR65, and a neuroblastoma cell line, CHP-212, dose-dependent inhibition of the G1/S phase and G2/M/G1 transitions was observed for both **11** and **12**.<sup>124–126</sup> S phase progression was also inhibited in a dose-dependent fashion. A complete cell cycle block was observed for both **11** and **12**, while Western blot analysis showed a decrease in CDK1 protein.<sup>127</sup> These cellular effects are consistent with CDK inhibition.



Olomoucine (**11**), roscovitine (**12**), and isopentenyladenine (**13**) have been cocrystallized with CDK2. For **12** the more potent *R*-isomer was employed. Interestingly, although the three compounds bind in the ATP pocket, the three compounds are oriented differently with respect to the protein, albeit the binding orientations of **12** and **11** are similar.<sup>127,128</sup> Key hydrogen bonds in **11** are N7 to NH of Leu-83, N6 to carbonyl of Leu-83, and OH of the amino alcohol to Gln-131 (Figure 5).<sup>73</sup>

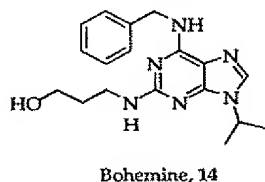




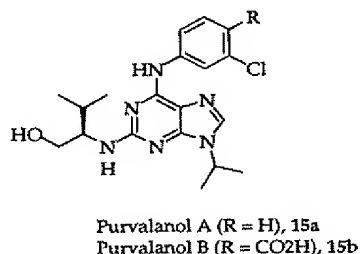
**Figure 6.** All atomic interactions between CDK2 and isopentenyladenine. Contacts with protein side chains are indicated by lines connecting to the respective residue box, while interactions to main chain atoms are shown as lines to the specific main chain atoms indicated. van der Waals contacts are indicated by dotted lines and hydrogen bonds by thick broken lines<sup>73</sup> (reproduced with permission).

Isopentenyladenine's (13) key interactions are N9 to Glu-81, N7 to Lys-33, and N3 to NH of Leu-83 (Figure 6).<sup>73</sup> The binding modes of **11** and **13** are twisted with respect to one another.<sup>121</sup>

Bohemine (**14**), a purine-based olomoucine analogue substituted with a 6-benzylamine and a 2-amino alcohol, is reported to have inhibitory effects on CDK1 (CDC2). Preclinical data for this molecule have been reported as showing a prolonged survival time in mice bearing B16 melanoma and P388D1 leukemia. Some animals with transplanted tumors were reported to have been cured when dosed with bohemine.<sup>130</sup>

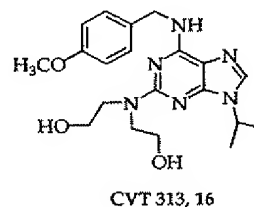


Attempts at maximizing the binding of the purine core have also employed a combinatorial approach. Recognizing that olomoucine binds within the ATP binding site (rotated 160° relative to ATP), elements of diversity were introduced at the 2-, 6-, and 9-positions in an attempt to enhance the competitive binding of the series, yielding the purvalanols (**15a,b**). Purvalanol B (**15b**) has an  $IC_{50}$  of 6 nM for CDK2/cyclin A. The compound shows selectivity with respect to CDK4/cyclin D1, erk1, PKC, and RAF in addition to other kinases. However, purvalanol was shown to be active for CDK2/cyclin E ( $IC_{50}$  = 9 nM) as well as CDK5/p35 ( $IC_{50}$  = 6 nM).<sup>131</sup>

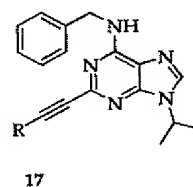


Other purine library approaches have resulted in the identification of CVT 313 (**16**),<sup>132–134</sup> a 2-,6-,9-trisubsti-

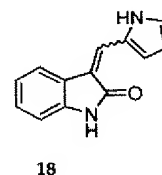
tuted purine which is a specific and ATP-competitive inhibitor of CDK2/cyclin A ( $IC_{50}$  = 0.5  $\mu$ M) with similar potency against CDK2/cyclin E. CDK4/cyclin D ( $IC_{50}$  = 215  $\mu$ M) and CDK1/cyclin B ( $IC_{50}$  = 4.2  $\mu$ M) inhibitory activity was less, and the compound did not inhibit PKC. Compound **16** reversibly inhibits cell proliferation at the G1/S or G2/M boundary and inhibits Rb hyperphosphorylation in MRC-5 cells. Compound **16** was shown to inhibit restenosis in rats exposed to a saline solution (1.25 mg/kg). Each animal treated showed at least 70% inhibition of the neointimal area. Purine **16** is being pursued in an antiproliferative strategy for the treatment of restenosis.<sup>135</sup>



In addition to these library approaches other groups have employed more traditional approaches in an effort to take advantage of the purine core to extend the SAR. 2-Alkynylated purines with terminal substituents including a number of alkyl alcohols (**17**) showed improved CDK1/cyclin B  $IC_{50}$ 's ranging from 0.18 to 1.2  $\mu$ M.<sup>136</sup>

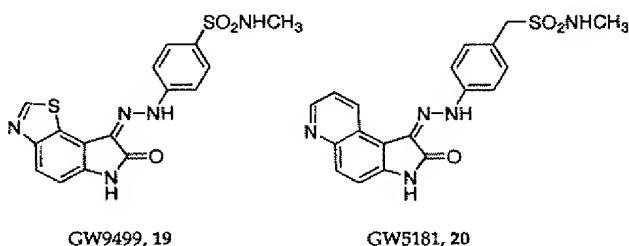


Oxindole **18** is a modestly potent CDK4/cyclin D-selective inhibitor (CDK4/cyclin D  $IC_{50}$  = 4.9  $\mu$ M, CDK2/cyclin E  $IC_{50}$  = 10.0  $\mu$ M, CDK1/cyclin B  $IC_{50}$  = 10.2  $\mu$ M). When tested in a human mammary adenocarcinoma cell line, MCF-7, the compound exhibited submicromolar potency ( $IC_{50}$  = 0.42  $\mu$ M). Interestingly, this oxindole showed potency in two Rb<sup>+</sup> cell lines (MCF-7 and ZR-75-1) and was less effective in an Rb<sup>-</sup> cell line (BT-549), a result consistent with CDK inhibition.<sup>137</sup>

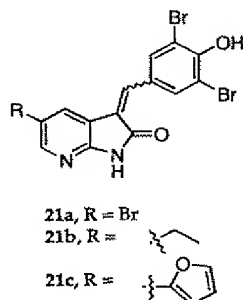


Two hydrazone analogues have been reported as potent CDK1 and CDK2 inhibitors. GW9499 (**19**) (CDK2  $IC_{50}$  = 3.5 nM) differs from GW5181 (**20**) (CDK2  $IC_{50}$  = 6.2 nM) in the third ring fusion of thiazole versus pyridine and a one-carbon truncation on the sulfonamide substituent.<sup>138,139</sup> Hydrazone **19** is the more potent compound in the cell lines reported, which include HDF, MD468, and RKO cell lines. When dosed at 5  $\mu$ M for 7 days, RKO cells exhibited cell death by day 4 of dosing while in MD468 cells, cell death was observed by day 6. When dosed once daily for 6 days at

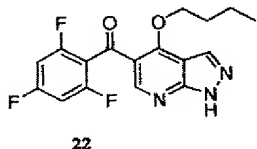
30 mg/kg iv in a RKO xenograft model, **19** showed little tumor growth inhibition. However, **20** dosed at higher amounts (100 mg/kg iv, once a day for 10 days) showed a modest decrease in tumor growth by day 20.<sup>140</sup>



A variation on this core incorporates a pyridine and is truncated by one ring as in **21**. A number of pyridine substitutions are reported including a 5-bromo, 5-ethyl, and 5-furyl moiety. The pendant aryl ring can also be substituted with alkyl or aryl groups. The listed compounds are reported to have CDK2 IC<sub>50</sub> values less than 1  $\mu$ M. This series does not always show CDK selectivity, a number of compounds in this series showing activity in a RAF assay (IC<sub>50</sub> < 1  $\mu$ M).<sup>141</sup>

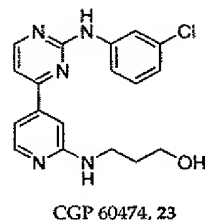


Pyrazolopyridines **22** were recently disclosed as CDK inhibitors. The most preferred compounds within this disclosure have a 4-butoxy substituent and a trifluoromethanone at the 5-position. The compounds are claimed to have enzyme IC<sub>50</sub> values of less than 50  $\mu$ M for CDK1/cyclin B, CDK2/cyclin E, and CDK4/cyclin D.<sup>142</sup>

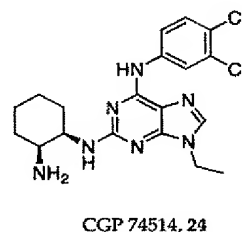


The phenylpyrimidine CGP 60474 (**23**) was found to be a potent inhibitor of both CDK1 (IC<sub>50</sub> = 0.020  $\mu$ M) and CDK2 (IC<sub>50</sub> = 0.05  $\mu$ M), while displaying weaker, submicromolar to low micromolar activity for PKC $\alpha$ , ERK-1, c-fgr, and CDK4. Other growth-related protein kinases were unaffected by exposure to CGP 60474 including JNK, p38, and other PKC subtypes. In the presence of 100 nM CGP 60474 FACS analysis of the cell cycle after release from the M/M block showed reversible G1/S arrest in U2-OS cells. U2-OS/Tag cells were arrested in the G2 phase. Interestingly, biochemical analysis of **23** revealed that no functional CDK/cyclin complexes were formed when the compound was present.<sup>143</sup> This result is very interesting as a compound

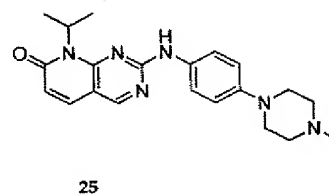
which is purely ATP-competitive should not change the conformation of the kinase sufficiently to prevent cyclin complexation. As such it may be that the compound is inhibiting other kinase targets which are themselves inhibiting CDK/cyclin complexation.



A second-generation compound from the purine series, CGP 74514 (**24**), is reported to be under preclinical evaluation. A potent inhibitor of CDK1 (IC<sub>50</sub> = 16 nM) and CDK2 (IC<sub>50</sub> = 9 nM), the compound showed weak activity for PKC (IC<sub>50</sub> = 6.1  $\mu$ M), EGFR (IC<sub>50</sub> = 9.3  $\mu$ M), and PKA (IC<sub>50</sub> = 125  $\mu$ M).<sup>144</sup> The biochemical analysis of this compound has not yet been reported.

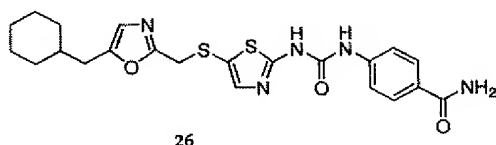


To date, only two series of compounds which show selectivity for CDK4/cyclin D1 over other kinases have been disclosed, the pyridopyrimidines and AG12275. A representative example of the pyridopyrimidine series is **25**, which was reported to have a CDK4/cyclin D IC<sub>50</sub> = 0.045  $\mu$ M. While selective with respect to CDK2/cyclin E (IC<sub>50</sub> = 0.27  $\mu$ M) and CDK1/cyclin B (IC<sub>50</sub> = 0.675  $\mu$ M) the compound was equipotent against CDK2/cyclin A (IC<sub>50</sub> = 0.058  $\mu$ M).<sup>145</sup>

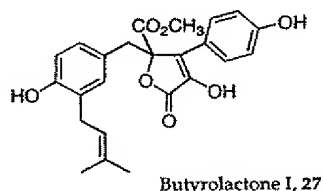


The structure of AG12275 has not been reported, but the compound is claimed to be selective for CDK4/cyclin D (IC<sub>50</sub> = 3.3 nM) as compared to CDK2/cyclin A (IC<sub>50</sub> = 220 nM) and CDK1/cyclin B (IC<sub>50</sub> = 325 nM). AG12286 has also been disclosed but does not have selectivity for CDK4/cyclin D (CDK4/cyclin D IC<sub>50</sub> = 12 nM, CDK2/cyclin A IC<sub>50</sub> = 5.7 nM, CDK1/cyclin B IC<sub>50</sub> = 2.2 nM). Both compounds are selective with respect to PKC, cAMP, ERK2, VEGF, and FGF. When dosed in an HCT cell line, AG12275 had an IC<sub>50</sub> = 300 nM, while in AG12286 had an IC<sub>50</sub> = 400 nM. In an in vivo HCT 116 xenograft model, AG12275 dosed at 50 mg/kg showed a 70% tumor growth inhibition at day 41.<sup>146</sup>

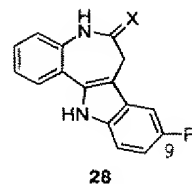
Thiazole **26** orients the core in a novel fashion with respect to the various chromophores and hydrogen-bonding groups. An amine linkage is required at the 2-position with ureas being claimed as preferred. There is a lipophilic moiety attached to the 4-position. This series of compounds is reported to have enzyme activity for CDK2/cyclin E, CDK4/cyclin D, and CDK1/cyclin B of less than 50  $\mu\text{M}$ .<sup>147</sup>



Butyrolactone I (**27**), reported to be a selective inhibitor of CDK2 and CDK1 kinase, was isolated from *Aspergillus* species F-25799. This ATP-competitive butyrolactone inhibited CDK1 ( $\text{IC}_{50} = 0.68 \mu\text{M}$ ) and CDK2 ( $\text{IC}_{50} = 0.82 \mu\text{M}$ ) yet did not inhibit MAPK, PKC, AK, or TyrK. This compound inhibited pRB phosphorylation at 0.48–31  $\mu\text{M}$  in selected nuclear extracts.<sup>148</sup> In metaphase-arrested *Xenopus* egg extracts pretreated with butyrolactone and then released into interphase, oscillation of DNA replication activity was prevented, thereby lengthening the cell cycle, a result consistent with the compound's CDK2 inhibitory activity.<sup>149</sup> The compound also showed antineoplastic effects on non-small-cell and small-cell lung cancer lines with  $\text{IC}_{50}$  values of around 50  $\mu\text{g/mL}$ . Additionally, the CDK1 kinase activity of PC-14 cells was inhibited by treatment with 20  $\mu\text{g/mL}$  of the lactone for 2 h. Cell cycle arrest was observed at the G2/M phase in a concentration-dependent manner, again consistent with CDK1/CDK2 inhibition.<sup>150</sup>



The paullones represent a series of benzazepines with submicromolar potencies for CDK1/cyclin B. The lead 9-bromo analogue **28** was reported to have a CDK1/cyclin B  $\text{IC}_{50}$  of 0.4  $\mu\text{M}$  while being selective with respect to CDK2/cyclin E ( $\text{IC}_{50} \sim 20 \mu\text{M}$ ) and CDK4/cyclin D1 (no inhibition observed). The compounds were found to be ATP-competitive and in serum-starved cells induced a G1 cell cycle arrest. Computer modeling suggested that the binding motif of these compounds is centered on a hydrogen bond through water to Phe-80 and a hydrogen bond to Leu-83 on the other side of the molecule. Interactions with Leu-134 and Isoleu-10 serve to maintain the core in the proper orientation. When tested in an HCT cell line the parent 9-bromo compound had an  $\text{IC}_{50} = 2.0 \mu\text{M}$ .<sup>151</sup> It should be noted that the CDK1/B used in analyzing this series was harvested from starfish oocytes. Although comparison of mammalian and starfish CDK1 shows them to be 75% identical and 85% homologous, it is not known if a direct comparison of enzyme inhibition is valid.<sup>152</sup>

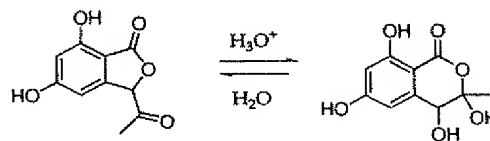


Cmpd	R	X	CDK1/B $\text{IC}_{50}$ ( $\mu\text{M}$ )
<b>28a</b>	Br	O	0.40
<b>28b</b>	H	O	7
<b>28c</b>	CN	O	0.024
<b>28d</b>	$\text{NO}_2$	O	0.035
<b>28e</b>	Br	S	2.3
<b>28f</b>	Br	S- $\text{CH}_3$	43

Further attempts to optimize the inhibitory activity of the 7,12-dihydroindolo[3,2-c][1]benzazepin-6(5*H*)-one series led to the observation that substitution at the 4-, 5-, 10-, 11-, and 12-positions leads to a decrease in enzyme inhibitory potency. An unalkylated ring amide was preferred as compared to a thiolactam, thioimide, or hydroxyamidine. Substitution of the bromo moiety by a nitro group (CDK1/cyclin B  $\text{IC}_{50} = 0.03 \mu\text{M}$ , HCT 116  $\text{IC}_{50} = 0.07 \mu\text{M}$ ) or by a cyano group (CDK1/cyclin B  $\text{IC}_{50} = 0.02 \mu\text{M}$ , HCT  $\text{IC}_{50} = 20 \mu\text{M}$ ) resulted in compounds with greater enzyme potencies but variable translation into cells.<sup>146</sup> Within this series the data shows that cellular activity does not parallel CDK activity, suggesting alternate mechanisms for cellular antiproliferative effects for some of the compounds.<sup>153</sup>

A variety of known anticancer compounds appear to have variable amounts of CDK inhibitory activity. Although it is not clear if this represents the mechanism of action for these compounds, it is of interest that CDK inhibitory effects are possible. Sodium butyrate, a product of colonic fermentation of dietary fiber, is one such CDK2 inhibitor. Cell cycle studies show cell proliferation is inhibited by this compound through a G1 phase block. Interestingly, CDK4 and CDK6 are unaffected by sodium butyrate. Studies have demonstrated that the observed CDK2 inhibition is effected by modulation of the cell cycle regulatory protein p21 and hence may be an indirect kinase interaction.<sup>154</sup>

Acetophthalidin (**29**) is a metabolite of the fungal strain BM923<sup>155</sup> and of *Ceratocystis minor*, a fungus associated with the blue stain disease of Canadian pine trees.<sup>156</sup> The compound has shown complete inhibition of cell cycle progression of mouse tsFT210 cells in the M phase at 6.25  $\mu\text{g/mL}$  concentrations.<sup>155</sup> The total synthesis of this compound<sup>157</sup> has been reported. It is not clear that the compound's antiproliferative effects are the direct result of CDK inhibition.



Acetophthalidin, **29**

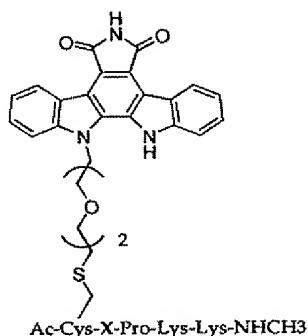
As cell biology becomes more defined, the molecular targets of currently marketed pharmaceutical compounds are frequently redefined. This is the case for the

known anticancer agent suramin which was shown to have some p34<sup>CDK1</sup> inhibitory activity with an  $IC_{50} = 4 \mu M$ . Additionally, the anticoagulant heparin also showed some CDK1 inhibitory activity ( $IC_{50} \sim 3.5 \mu g/mL$ ).<sup>158</sup> Although these compounds were reported to be inhibitors of CDK1, their potency is weak.

Also included in this series are select selenium compounds which have found use as anticancer agents but for which the mechanism of action was not always defined.<sup>159,160</sup> One representative compound in this class, methylselenocysteine, has been shown to inhibit CDK2 activity in synchronized mouse mammary epithelial tumor cells while having no impact on CDK4 activity. PKC activity in these cells was not reported. Methylselenocysteine arrested cells in the S phase during the TM6 cell cycle.<sup>161</sup>

Small synthetic peptides have also proven to have in vitro efficacy. A 20-amino acid residue corresponding to residues 84–103 of p16 has been claimed to interact with CDK4 and CDK6, inhibiting CDK4.<sup>162</sup> This sort of peptide approach is limited for producing pharmaceutical entities; however, peptides represent interesting possibilities as templates for the design of non-ATP-competitive small molecules.

There has been one report of an attempt to combine an ATP-competitive approach with a peptide binding site approach (30). Here, a bisindolylmaleimide was utilized as a core unit to which a pseudopeptide was linked through an ether tether.<sup>163</sup> This approach was effective at affording modest inhibitors of CDK1, the in vitro  $IC_{50}$  being best when the pseudopeptide was CPKK ( $IC_{50} = 4.5 \mu M$ ). The compounds in this series were shown to have some selectivity when compared to PKA, PTK, CAMK, and EGFR. The compounds, however, do not show strong selectivity with respect to PKC and are very modest inhibitors of CDK1.



30

## Conclusion

Research into the inhibition of CDKs has increased over the last 5 years. However, our understanding of the biology and the structural features required to selectively mediate cell progression is still in its infancy.

CDKs have received most attention as potential targets for cancer therapy. The frequency of mutation in the Rb kinase pathway in human tumors has focused attention on the G1 kinases, in particular cyclin D1/CDK4, as potential therapeutic targets. Cyclin D1 expression is upregulated in several tumor types, most

notably breast cancer.<sup>164–166</sup> CDK4 has been shown to be mutated to a form resistant to the natural inhibitor p16 in at least one melanoma kindred,<sup>167</sup> and the CDK4 locus is amplified in a subset of sarcomas.<sup>168</sup> Furthermore, alterations in the p16<sup>INK4</sup> locus are very common in human cancer patients.<sup>169,170</sup>

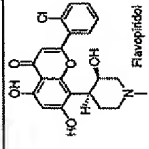
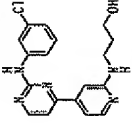
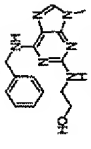
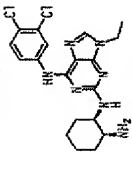
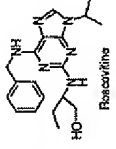
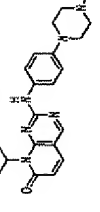
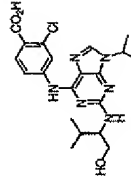
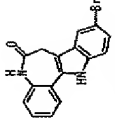
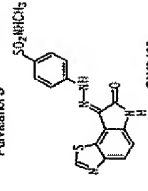
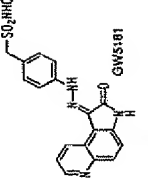
Recent discoveries, however, suggest a reevaluation of the importance of CDK4-targeted therapies is warranted.<sup>171</sup> First, the CDK4 knockout mouse is viable with limited pancreatic<sup>172</sup> and reproductive defects.<sup>173</sup> CDK4 dominant negative constructs are unable to halt cell cycle progression.<sup>174</sup> Second, cell cycle progression is unaffected in cells where cyclin D/CDK activity is undetectable.<sup>173,175</sup> However, quiescent CDK4  $-/-$  fibroblasts show delayed S phase entry upon serum stimulation likely due to increased p27 binding to cyclin E/CDK2.<sup>173</sup> Third, the importance of p16 as a tumor suppressor protein no longer points so clearly to cyclin D/CDKs as preferred targets. Although p16 interacts specifically with CDK4 and CDK6, this interaction displaces p21 and p27 which serve an assembly function for cyclin D/CDK complexes. Displaced p21 and p27 can inhibit other cyclin/CDKs. Thus p16 expression can effectively inhibit multiple cyclin/CDKs.<sup>176</sup> Furthermore, cyclin D overexpression by providing, together with CDK4, a sink for p21/p27 could effectively indirectly activate multiple CDKs. Finally, the p16 locus itself encodes two gene products: p16 and p14/p19 ARF.<sup>177–179</sup> p16 inhibits cyclin/CDKs; p14/p19 ARF inhibits mdm2 function and impacts the p53 pathway.<sup>180–184</sup>

Cyclin D1/CDK4, therefore, has at least two activities: an enzymatic activity associated with Rb phosphorylation and a sequestration activity for negative cell cycle regulators such as p27. Thus, the critical role of cyclin D/CDK4,6 enzymatic activity in normal cell cycle progression is currently open to question. Granted, no one has yet determined whether this enzyme activity may have a more central role in the transformed state. Nevertheless, current data suggests the possibility that a selective inhibitor of cyclin D/CDK4 enzymatic activity may be ineffective at halting cell cycle progression.

Conversely, cyclin/CDK2 complexes appear to play a more critical role in the cell cycle. No CDK2 knockout mouse has been reported, but unlike the case for CDK4, CDK2 dominant negative constructs can halt cell cycle progression.<sup>174</sup> Unlike cyclin D knockout animals, the cyclin A knockout mouse is embryonic lethal.<sup>185</sup> Importantly, from the point of view of cancer therapy, signals for anchorage-independent growth, a key feature of tumor cells, are mediated through CDK2 complexes.<sup>186,187</sup> Finally, a test for CDK2 inhibition as a therapeutic approach to cancer was recently reported by Chen and co-workers.<sup>188</sup> They reported that a peptide inhibitor of CDK2 function was selectively able to kill transformed versus normal cells. It is important to realize that this peptide inhibitor, although able to inhibit the kinase, did not target the ATP binding site on CDK2 but rather a site on the cyclin subunit, important for binding and down-modulation of E2F.

Thus, the current understanding of the cell cycle has focused on CDK2 as a preferred CDK therapeutic target. However, what these recent advances also point out is that we still have much to learn about the regulatory balancing act that controls orderly cell cycle progression

Table 2. Key CDK Inhibitors

Inhibitor	CDK1/B IC <sub>50</sub> (μM)	CDK2/A IC <sub>50</sub> (μM)	CDK2/E IC <sub>50</sub> (μM)	CDK4/D IC <sub>50</sub> (μM)	Inhibitor	CDK1/B IC <sub>50</sub> (μM)	CDK2/A IC <sub>50</sub> (μM)	CDK2/E IC <sub>50</sub> (μM)	CDK4/D IC <sub>50</sub> (μM)
 Flavopiridol	0.4	0.1	--	0.4	 CGP8474	0.020	--	0.050	--
 Okadaic acid	7.0	--	7.0	inactive	 CGP74514	0.016	--	0.009	0.029
 Roscovitine	0.65	--	--	--	 AG12286	0.675	0.058	0.27	0.045
 Purvalan 8	--	0.006	0.009	--	 NO <sub>2</sub> -Paullone	0.035*	--	--	--
 GW9409	--	--	0.0035	--	AG12286	0.0022	0.0057	--	0.012
 GW5101	--	--	0.0062	--	AG12275	0.325	0.220	--	0.0033

\*CDK2 harvested from starfish oocytes.

and exit. Hopefully, selective chemical kinase inhibitors will provide not only useful therapeutics in the near term but also tools to understand the cycle itself. With this increased understanding, maybe we can drive discovery toward yet more selective and effective therapies.

There are many challenges in the development of small molecule inhibitors of the CDKs, as evidenced from some of the key compounds presented in this Perspective (Table 2). To achieve success in the pharmaceutical modulation of the cell cycle, two main issues must be explored.

First, selectivity among the kinases for efficacious and safe control of the cell cycle has not been realized. All kinases have an ATP binding pocket which tends to have a high degree of homology. There are an estimated 2000 kinases in the human body.<sup>189</sup> As the vast majority of reported compounds are ATP-competitive, the synthesis of CDK inhibitors with good selectivity among the kinases remains a continuing challenge. A truly selective CDK inhibitor has not yet been realized, and the level of selectivity that would be required of a clinically successful inhibitor has not been and will not be defined until more selective inhibitors are evaluated.

Second, as evidenced from those compounds with nanomolar potency for the CDK enzymes, two hydrogen bonds between the substrate and the cyclin-dependent kinase in the ATP pocket are preferred. As there have been no compounds disclosed with picomolar potency, it remains a possibility that an additional binding interaction will be key in achieving better potency and selectivity.

A wide spectrum of cellular activity is observed for the molecules presented in this Perspective. This variability is possibly the result of a number of combined factors including variable cell penetration, binding to media, and substrate competition. However, trends for the successful inhibition of CDKs are becoming apparent. Key structural hydrogen-bonding motifs are seen repeatedly for compounds which bind in the ATP pocket. These binding motifs are seen for the more potent analogues including flavopiridol, olomoucine, and roscovitine. As our understanding of inhibitor binding, cellular activity, and ultimately the correlation to in vivo activity becomes refined, our approaches to modulating the cell cycle will improve.

## Biographies

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## References

- (1) Foye, W. O. *Cancer Chemotherapeutic Agents*; American Chemical Society: Washington, DC, 1995.
- (2) Rowinsky, E. K. The Development and Clinical Utility of the Taxane Class of Antimicrotubule Chemotherapy Agents. *Annu. Rev. Med.* 1997, 48, 353-374.
- (3) Takimoto, C. H.; Wright, J.; Arbuck, S. G. Clinical Applications of the Camptothecins. *Biochim. Biophys. Acta* 1998, 1400, 107-119.
- (4) Ayril-Kaloustian, S.; Skotnicki, J. S. Ras Farnesyltransferase Inhibitors. *Annu. Rep. Med. Chem.* 1996, 31, 171-180.
- (5) Omura, S.; Sasaki, Y.; Iwai, Y.; Takeshima, H. Staurosporine, a Potentially Important Gift from a Microorganism. *J. Antibiot.* 1995, 48, 535-548.
- (6) Yasuzawa, T.; Lida, T.; Yoshida, M.; Hirayama, N.; Takahashi, M.; Shirahata, K.; Sano, H. The Structures of the Novel Protein Kinase C Inhibitors K-252a, b, c and d. *J. Antibiot.* 1986, 39, 1072-1078.
- (7) Fry, D. W.; Kraker, A. J.; McMichael, A.; Ambrosio, L. A.; Nelson, J. M.; Leopold, W. R.; Connors, R. W.; Bridges, A. J. A Specific Inhibitor of the Epidermal Growth Factor Receptor Tyrosine Kinase. *Science* 1994, 265, 1093-1095.
- (8) Fry, D. W. Recent Advances in Tyrosine Kinase Inhibitors. *Annu. Rep. Med. Chem.* 1996, 31, 151-160.
- (9) Garrett, M. D.; Fattaey, A. CDK Inhibition and Cancer Therapy. *Curr. Opin. Genet. Dev. (England)* 1999, 9, 104-111.
- (10) Webster, K. R. The Therapeutic Potential of Targeting the Cell Cycle. *Exp. Opin. Invest. Drugs* 1998, 7, 865-887.
- (11) Lees, J. A.; Weinberg, R. A. Tossing Monkey Wrenches into the Clock: New Ways of Treating Cancer. *Proc. Natl. Acad. Sci. U.S.A.* 1999, 96, 4221-4223.
- (12) Hunter, T.; Pines, J. Cyclins and Cancer II: Cyclin D and CDK Inhibitors Come of Age. *Cell* 1994, 79, 573-583.
- (13) Pines, J. Cyclins and Cyclin-Dependent Kinases: Theme and Variations. *Adv. Cancer Res.* 1995, 55, 181-212.
- (14) Planas-Silva, M. D.; Weinberg, R. A. The Restriction Point and Control of Cell Proliferation. *Curr. Opin. Cell Biol.* 1997, 9, 768-772.
- (15) Murray, A. Cyclin-Dependent Kinases: Regulators of the Cell Cycle and More. *Chem. Biol.* 1994, 1, 191-195.
- (16) Kaldis, P. The CDK-Activating Kinase (CAK): From Yeast to Mammals. *Cell. Mol. Life Sci.* 1999, 55, 284-296.
- (17) Fisher, R. P.; Morgan, D. O. CAK in TFIIF: Crucial Connection or Confounding Coincidence? *Biochim. Biophys. Acta* 1996, 1288, 7-10.
- (18) Tsai, L. H.; Delalle, I.; Caviness Jr., V. S.; Chae, T.; Harlow, E. p35 is a Neural-Specific Regulatory Subunit of Cyclin-Dependent Kinase 5. *Nature* 1994, 371, 419-423.
- (19) Lew, J.; Huang, Q. Q.; Winkler, R. J.; Aebersold, R.; Hunt, T.; Wang, J. H. MRC Group in Signal Transduction, University of Calgary, Alberta, Canada. A Brain-Specific Activator of Cyclin-Dependent Kinase 5. *Nature* 1994, 371, 423-426.
- (20) Dou, Q. P.; Levin, A. H.; Zhao, S. Z.; Pardee, A. B. Cyclin E and Cyclin A as Candidates for the Restriction Point Protein. *Cancer Res.* 1993, 53, 1493-1497.
- (21) Zetterberg, A.; Larsson, O.; Womn, K. G. What is the restriction point? *Curr. Opin. Cell Biol.* 1995, 7, 835-842.
- (22) Dowdy, S. F.; Hinds, P. W.; Louie, K.; Reed, S. I.; Arnold, A.; Weinberg, R. A. Physical Interaction of the Retinoblastoma Protein with Human D Cyclins. *Cell* 1993, 73, 499-511.
- (23) Pan, W.; Sun, T.; Hoess, R.; Grafstrom, R. Defining the Minimal Portion of the Retinoblastoma Protein that Serves as an Efficient Substrate for CDK4 Kinase/Cyclin D1 Complex. *Carcinogenesis* 1998, 19, 765-769.
- (24) Luo, R. X.; Postigo, A. A.; Dean, D. C. Rb Interacts with Histone Deacetylase to Repress Transcription. *Cell* 1998, 92, 463-473.
- (25) Chellappan, S.; Hiebert, S.; Mudryi, M.; Horowitz, J. M.; Nevins, J. R. The E2F Transcription Factor is a Cellular Target for the RB Protein. *Cell* 1991, 65, 1053-1061.
- (26) Brown, V. D.; Phillips, R. A.; Gallie, B. L. Cumulative Effect of Phosphorylation of pRB on Regulation of E2F Activity. *Mol. Cell. Biol.* 1999, 19, 3246-3256.
- (27) Sherr, C. J. Cancer Cell Cycles. *Science* 1996, 274, 1672-1677.
- (28) Jackson, P. K.; Chevalier, S.; Philippe, M.; Kirschner, M. W. Early Events in DNA Replication Require Cyclin E and are Blocked by p21CIP1. *J. Cell. Biol.* 1995, 130, 755-769.
- (29) Zou, L.; Stillman, B. Formation of a Preinitiation Complex by S-phase Cyclin CDK-Dependent Loading of Cdc45p onto Chromatin. *Science* 1998, 280, 593-596.



- (30) Bartek, J.; Bartkova, J.; Lukas, J. The Retinoblastoma Protein Pathway and the Restriction Point. *Curr. Opin. Cell Biol.* **1996**, *8*, 5–14.
- (31) Medema, R. H.; Herrera, R. E.; Lam, F.; Weinberg, R. A. Growth suppression by p16<sup>ink4a</sup> Requires Functional Retinoblastoma Protein. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 6289–6293.
- (32) Cheng, M.; Olivier, P.; Diehl, J. A.; Fero, M.; Roussel, M. F.; Roberts, J. M.; Sherr, C. J. The p21<sup>cip1</sup> and p27<sup>kip1</sup> CDK "Inhibitors" are Essential Activators of Cyclin D-Dependent Kinases in Murine Fibroblasts. *EMBO J.* **1999**, *18*, 1571–1583.
- (33) Rane, S. G.; Dubus, P.; Mettus, R. V.; Galbreath, E. J.; Boden, G.; Reddy, E. P.; Barbacid, M. Loss of CAK4 Expression Causes Insulin-Deficient Diabetes and CAK4 Activation Results in  $\beta$ -Islet Cell Hyperplasia. *Nat. Genet.* **1999**, *22*, 44–52.
- (34) Lukas, J.; Bartkova, J.; Bartek, J. Convergence of Mitogenic Signaling Cascades from Diverse Classes of Receptors at the Cyclin D-Cyclin-Dependent Kinase: pRb-Controlled G1 Checkpoint. *Mol. Cell Biol.* **1996**, *16*, 6917–6925.
- (35) Lacey, K.; Jackson, P. K.; Stearns, T. Cyclin-Dependent Kinase Control of Centrosome Duplication. *Natl. Acad. Sci. U.S.A.* **1999**, *96*, 2817–2822.
- (36) Krude, T.; Jackman, M.; Pines, J.; Laskey, R. A. Cyclin/Cdk-Dependent Initiation of DNA Replication in a Human Cell-Free System. *Cell* **1997**, *88*, 109–119.
- (37) Morgan, D. O.; De Bondt, H. L. Protein Kinase Regulation: Insights from Crystal Structure Analysis. *Curr. Opin. Cell Biol.* **1994**, *6*, 239–246.
- (38) Berry, L. D.; Coult, K. L. Regulation of Cdc2 Activity by Phosphorylation at T14/Y15. *Prog. Cell Cycle Res.* **1996**, *2*, 99–105.
- (39) Lowe, M.; Rabouille, C.; Nakamura, N.; Watsch, R.; Jackson, M.; Tamsa, E.; Rahman, D.; Pappin, D. J. C.; Warren, G. Cdc2 Kinase Directly Phosphorylates the Cis-Golgi Matrix Protein GM130 and is Required for Golgi Fragmentation in Mitosis. *Cell* **1998**, *94*, 783–793.
- (40) Morgan, D. O. Principles of CDK Regulation. *Nature* **1995**, *375*, 131–134.
- (41) De Bondt, H. L.; Rosenblatt, J.; Jancarik, J.; Jones, H. D.; Morgan, D. O.; Kim, S. H. Cyclins and Cyclin Dependent Kinases. *Nature* **1993**, *363*, 595–602.
- (42) Kelly, B. L.; Wolfe, K. G.; Roberts, J. M. Identification of a Substrate-Targeting Domain in Cyclin E for Phosphorylation of the Retinoblastoma Protein. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2535–2540.
- (43) Hinz, M.; Krappmann, D.; Eichten, A.; Heder, A.; Scheidereit, C.; Strauss, M. NF- $\kappa$ B Function in Growth Control: Regulation of Cyclin D1 Expression and G<sub>0</sub>/G<sub>1</sub>-to-S-Phase Transition. *Mol. Cell Biol.* **1999**, *19*, 2690–2698.
- (44) King, R. W.; Deshaies, R. J.; Peters, J.; Kirschner, M. W. How Proteolysis Drives the Cell Cycle. *Science* **1996**, *274*, 1652–1658.
- (45) Yee, A.; Nichols, M. A.; Wu, L.; Hall, F. L.; Kobayashi, R.; Xiong, Y. Molecular Cloning of CDK7-associated Human MAT1, a Cyclin-Dependent Kinase-Activating Kinase (CAK) Assembly Factor. *Cancer Res.* **1995**, *55*, 6058–6062.
- (46) Gerber, M. R.; Farrell, A.; Deshaies, R. J.; Herskowitz, I.; Morgan, D. O. Cdc37 is Required for Association of the Protein Kinase cdc28 with G1 and Mitotic Cyclins. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 4651–4655.
- (47) Fattaey, A.; Harkow, E. New Functional Activities for the p21 Family of CDK Inhibitors. *Genes Dev.* **1997**, *11*, 847–862.
- (48) Fisher, R. P.; Morgan, D. O. *Cell* **1994**, *78*, 713–724.
- (49) Atherton-Fessler, S.; Hannig, G.; Piwnicka-Worms, H. Reversible Tyrosine Phosphorylation and Cell Cycle Control. *Semin. Cell Biol.* **1993**, *4*, 433–442.
- (50) Fattaey, A.; Booher, R. N. Myt1: A Wee1-Type Kinase that Phosphorylates Cdc2 on Residue Thr14. *Prog. Cell Cycle Res.* **1997**, *3*, 233–240.
- (51) Draetta, G.; Eckstein, J. Cdc25 Protein Phosphatases in Cell Proliferation. *Biochim. Biophys. Acta* **1997**, *1332*, M53–M63.
- (52) Hoffman, I.; Karsenti, E. The Role of cdc25 in Checkpoints and Feedback Controls in the Eukaryotic Cell Cycle. *J. Cell. Sci. Suppl.* **1994**, *18*, 75–79.
- (53) Sherr, C. J.; Roberts, J. M. Inhibitors of Mammalian G<sub>1</sub> Cyclin-Dependent Kinases. *Genes Dev.* **1995**, *9*, 1149–1163.
- (54) Serrano, M.; Hannon, G. J.; Beach, D. A New Regulatory Motif in Cell-Cycle Control Causing Specific Inhibition of Cyclin D/CDK4. *Nature* **1993**, *366*, 704–707.
- (55) McConnell, B. B.; Gregory, F. J.; Stott, F. J.; Hara, E.; Peters, G. Induced Expression of p16<sup>ink4a</sup> Inhibits Both CDK4- and CDK2-Associated Kinase Activity by Reassortment of Cyclin-CDK Inhibitor Complexes. *Mol. Cell Biol.* **1999**, *19*, 1981–1989.
- (56) Hartwell, L. H.; Weinart, T. A. Checkpoints: Controls that Ensure the Order of Cell Cycle Events. *Science* **1989**, *246*, 629–634.
- (57) Reed, S. I. Control of the G<sub>1</sub>/S Transition. *Cancer Surv.* **1997**, *29*, 7–23.
- (58) Tournet, J.; McKeon, F. Tyrosine Kinases Wee1 and Mik1 as Effectors of DNA Replication Checkpoint Control. *Prog. Cell Cycle Res.* **1996**, *2*, 91–97.
- (59) Boddy, M. N.; Furnari, B.; Mondesert, O.; Russell, P. Replication Checkpoint Enforced by Kinases Cds1 and Chk1. *Science* **1998**, *280*, 909–912.
- (60) Huang, M.; Zhou, Z.; Elledge, S. J. The DNA Replication and Damage Checkpoint Pathways Induce Transcription by Inhibition of the Crt1 Repressor. *Cell* **1998**, *94*, 595–605.
- (61) Zeng, Y.; Forbes, K. C.; Wu, Z.; Moreno, S.; Piwnicka-Worms, H.; Enoch, T. Replication of Checkpoint Requires Phosphorylation of the Phosphates Cdc25 by Cds1 or Chk1. *Nature* **1998**, *395*, 507–510.
- (62) Amon, A. The Spindle Checkpoint. *Curr. Opin. Genet. Dev.* **1999**, *9*, 69–75.
- (63) Hardwick, K. G. The Spindle Checkpoint. *Trends Genet.* **1998**, *14*, 1–4.
- (64) Millard, S. S.; Koff, A. Cyclin-Dependent Kinase Inhibitors in Restriction Point Control, Genomic Stability and Tumorigenesis. *J. Cell. Biochem. Suppl.* **1998**, *30*, 37–42.
- (65) DelSal, G.; Loda, M.; Pagano, M. Cell Cycle and Cancer: Critical Events at the G1 Restriction Point. *Crit. Rev. Oncogene* **1996**, *7*, 127–142.
- (66) Sherr, C. J. Cancer Cell Cycles. *Science* **1996**, *274*, 1672–1677.
- (67) Hartwell, L. H.; Kastan, M. B. Cell Cycle Control and Cancer. *Science* **1994**, *266*, 1821–1828.
- (68) Hall, M.; Peters, G. Genetic Alterations of Cyclins, Cyclin-Dependent Kinases, and Cdk Inhibitors in Human Cancer. *Adv. Cancer Res.* **1996**, *56*, 67–108.
- (69) Heintz, N. Cell Death and the Cell Cycle: A Relationship Between Transformation and Neurodegeneration? *Trends Biochem. Sci.* **1993**, *18*, 157–159.
- (70) Arendt, T.; Holzer, M.; Gartner, U.; Bruckner, M. K. Aberrancies in Signal Transduction and Cell Cycle Related Events in Alzheimer's Disease. *J. Neural Transm. Suppl.* **1998**, *54*, 147–158.
- (71) Wei, P.; Garber, M. E.; Fang, S.; Fischer, W. H.; Jones, K. A. A Novel CDK9-Associated C-Type Cyclin Interacts Directly with HIV-1 Tat and Mediates Its High-Affinity, Loop-Specific Binding to TAR RNA. *Cell* **1998**, *92*, 451–462.
- (72) Morgan, D. O. The Dynamics of Cyclin Dependent Kinase Structure. *Curr. Opin. Cell Biol.* **1996**, *8*, 767–772.
- (73) Kim, S.-H. Structure-based Inhibitor Design for CDK2, a Cell Cycle Controlling Protein Kinase. *Pure Appl. Chem.* **1998**, *70*, 555–565.
- (74) De Bondt, H. L.; Rosenblatt, J.; Jancarik, J.; Jones, H. D.; Morgan, D. O.; Kim, S.-H. Crystal Structure of Cyclin Dependent Kinase 2. *Nature* **1993**, *363*, 595–602.
- (75) Jeffrey, P. D.; Russo, A. A.; Polyak, K.; Gibbs, E.; Hurwitz, J.; Massague, J.; Pavletich, N. P. Mechanism of CDK Activation Revealed by the Structure of a Cyclin A-CDK2 Complex. *Nature* **1995**, *376*, 313–320.
- (76) Meijer, L.; Kim, S. H. Chemical Inhibitors of Cyclin-Dependent Kinases. *Methods Enzymol.* **1997**, *283*, 113–128.
- (77) Mayrose, D. R.; Nichols, M. A.; Xiong, Y.; Ke, H. Purification and Crystallization of Cyclin-dependent Kinase Inhibitor p21. *Protein Sci.* **1996**, *5*, 1928–1930.
- (78) Luh, F. Y.; Archer, S. J.; Domaille, P. J.; Smith, B. O.; Owen, D.; Brotherton, D. H.; Raine, A. R. C.; Xu, X.; Brizuela, L.; Brenner, S.; Laue, E. D. Structure of the Cyclin-Dependent Kinase Inhibitor p19<sup>ink4d</sup>. *Nature* **1997**, *389*, 999–1003.
- (79) Brotherton, D. H.; Dhanaraj, V.; Wick, S.; Brizuela, L.; Domaille, P. J.; Volyanik, E.; Xu, X.; Parisini, E.; Smith, B. O.; Archer, S. J.; Serrano, M.; Brenner, S. L.; Blundell, T. L.; Laue, E. D. Crystal Structure of the Complex of the Cyclin D-dependent kinase CDK6 Bound to the Cell Cycle Inhibitor p19<sup>INK4d</sup>. *Nature* **1998**, *395*, 244–250.
- (80) Fong, T. A. T.; Shawver, L. K.; Sun, L.; Tang, C.; App, H.; Powell, T. J.; Kim, Y. H.; Schreck, R.; Wang, X.; Risau, W.; Ullrich, A.; Hirth, K. P.; McMahon, G. SU5416 is a Potent and Selective Inhibitor of the Vascular Endothelial Growth Factor Receptor (Flk-1/KDR) that Inhibits Tyrosine Kinase Catalysis, Tumor Vascularization, and Growth of Multiple Tumor Types. *Cancer Res.* **1999**, *59*, 99–106.
- (81) *R&D Focus* **1997**, Dec 15.
- (82) Singh, P.; Kumar, R. Inhibitors of the Epidermal Growth Factor Receptor Protein Tyrosine Kinase. A Quantitative Structure-Activity Relationship Analysis. *J. Enzyme Inhib.* **1998**, *13*, 125–134.
- (83) Gibson, K. H.; Brundy, W.; Godfrey, A. A.; Woodburn, J. R.; Ashton, S. E.; Curry, B. J.; Scarlett, L.; Barker, A. J.; Brown, D. S. Epidermal Growth Factor Receptor Tyrosine Kinase: Structure-Activity Relationships and Antitumor Activity of Novel Quinazolines. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2723–2728.
- (84) Thompson, A. M.; Murray, D. K.; Elliott, W. L.; Fry, D. W.; Nelson, J. A.; Showalter, H. D. Hollis; Roberts, B. J.; Vincent, P. W.; Denny, W. A. Tyrosine Kinase Inhibitors. 13. Structure-

- Activity Relationships for Soluble 7-Substituted 4-[(3-Bromo-phenyl)amino]pyrido[4,3-d]pyrimidines Designed as Inhibitors of the Tyrosine Kinase Activity of the Epidermal Growth Factor Receptor. *J. Med. Chem.* **1997**, *40*, 3915–3925.
- (85) Bencteux, E.; Houssin, R.; Henichart, J. A short and Unequivocal Synthesis of 5-Aminotetrazolo[1,5-a]quinazoline as a Tricyclic Analogue of 4-(3-Bromoanilino)-6,7-dimethoxyquinazoline (PD 153035). *J. Heterocycl. Chem.* **1997**, *34*, 1375–1378.
- (86) Myers, M. R.; Setzer, N. N.; Spada, A. P.; Persons, P. E.; Ly, C. Q.; Maguire, M. P.; Zulli, A. L.; Cheney, D. L.; Zilberstein, A.; Johnson, S. E.; Franks, C. F.; Mitchell, K. J. The Synthesis and SAR of New 4-(N-alkyl-N-phenyl)amino-6,7-dimethoxyquinazolines and 4-(N-alkyl-N-phenyl)aminopyrazolo[3,4-d]pyrimidines, Inhibitors of CSF-1R Tyrosine Kinase Activity. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 421–424.
- (87) Myers, M. R.; Setzer, N. N.; Spada, A. P.; Zulli, A. L.; Hsu, C. J.; Zilberstein, A.; Johnson, S. E.; Hook, L. E.; Jacoski, M. V. The Preparation and SAR of 4-(Anilino), 4-(Phenoxy), and 4-(Thiophenoxy)-Quinazolines: Inhibitors of p56lck and EGF-R Tyrosine Kinase Activity. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 417–420.
- (88) Newcastle, G. W.; Palmer, B. D.; Bridges, A. J.; Showalter, H. D. H.; Sun, L.; Nelson, J.; McMichael, A.; Kraker, A. J.; Fry, D. W.; Denny, W. A. Tyrosine Kinase Inhibitors. 9. Synthesis and Evaluation of Fused Tricyclic Quinazoline Analogues as ATP Site Inhibitors of the Tyrosine Kinase Activity of the Epidermal Growth Factor Receptor. *J. Med. Chem.* **1996**, *39*, 918–928.
- (89) *R&D Focus* **1997**, Aug 11.
- (90) Furusaki, A.; Hashiba, N.; Matsumoto, T.; Hirano, A.; Iwai, Y.; Omura, S. The Crystal and Molecular Structure of Staurosporine, a New Alkaloid from a Streptomyces Strain. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 3681–3685.
- (91) Meijer, L. Chemical Inhibitors of Cyclin-Dependent Kinases. *Trends Cell. Biol.* **1996**, *6*, 393–397.
- (92) Gadbois, D.; Hamaguchi, J. R.; Swank, R. A.; Bradbury, E. M. Staurosporine is a Potent Inhibitor of p34cdc2 and p34cdc2-like Kinases. *Biochem. Biophys. Res. Commun.* **1992**, *184*, 80–85.
- (93) Meijer, L. Chemical Inhibitors of Cyclin-Dependent Kinases. In *Progress in Cell Cycle Research*; Meijer, L., Guidet, S., Tung, H. Y. L., Eds.; Plenum Press: New York, 1995; Vol. 1, pp 351–361.
- (94) Lawrie, A. M.; Noble, M. E. M.; Tunnah, P.; Brown, N. R.; Johnson, L. N.; Endicott, J. A. Protein Kinase Inhibition by Staurosporine Revealed in Details of the Molecular Interaction with CDK2. *Nat. Struct. Biol.* **1997**, *4*, 796–801.
- (95) *Drug and Market Development* **1997**, May 1, 98–100.
- (96) Urano, M.; Reynolds, R.; Begley, J. Protein kinase C Inhibitor as a Potent Inhibitor of Accelerated Repopulation During Radiotherapy: I. Growth Inhibitory, Cytotoxic, and Indirect Radiosensitizing Effects of a Staurosporine Derivative (UCN-01) In Vitro. *Radiat. Oncol. Invest.* **1995**, *3*, 64–71.
- (97) Wang, Q.; Fan, S.; Eastman, A.; Worland, P. J.; Sausville, E. A.; O'Connor, P. M. UCN-01: A Potent Abrogator of G2 Checkpoint Function in Cancer Cells with Disrupted p53. *J. Natl. Cancer Inst.* **1996**, *88*, 956–965.
- (98) Akinaga, S.; Nomura, K.; Gomi, K.; Okabe, M. Enhancement of Antitumor Activity of Mitomycin C In Vitro and In Vivo by UCN-01, a Selective Inhibitor of Protein Kinase C. *Cancer Chemother. Pharmacol.* **1993**, *32*, 183–189.
- (99) Seynaeve, C. M.; Stedler, S. M.; Sebers, S.; Kaur, G.; Sausville, E. A.; Worland, P. J. Cell Cycle Arrest and Growth Inhibition by the Protein Kinase Antagonist UCN-01 in Human Breast Carcinoma. *Cancer Res.* **1993**, *53*, 2081–2086.
- (100) Bredel, M.; Pollack, I. F.; Freund, J. M.; Rusnak, J.; Lazo, J. S. 7-Hydroxystaurosporine (UCN-01), a Selective Inhibitor of Protein Kinase C, Blocks Malignant Glioma Growth by Inducing Apoptosis Independent of p53 and in a Time-Dependent manner. *Proc. Am. Assoc. Cancer Res.* **1997**, *38*, 500.
- (101) Schwartz, G. K.; Farsi, K.; Danso, D.; Dhupar, S. K.; Kelsen, D.; Spriggs, D. The Protein Kinase C (PKC) Inhibitors UCN-01 and Flavopiridol (FLAVO) Significantly Enhance the Cytotoxic Effect of Chemotherapy by Promoting Apoptosis in Gastric and Breast Cancer Cells. *Proc. ASCO* **1996**, *15*, 501.
- (102) Link, J. T.; Raghavan, S.; Gallant, M.; Danishefsky, S. J.; Chou, T. C.; Ballas, L. M. Staurosporine and ent-Staurosporine: The First Total Syntheses, Prospects for a Regioselective Approach, and Activity Profiles. *J. Am. Chem. Soc.* **1996**, *118*, 2825–2842.
- (103) Pereira, E. R.; Belin, L.; Sancelme, M.; Prudhomme, M.; Ollier, M.; Rapp, M.; Severe, D.; Riou, J.-F.; Fabbro, D.; Meyer, T. Structure–Activity Relationships in a Series of Substituted Indolocarbazoles: Topoisomerase I and Protein Kinase C Inhibition and Antitumoral and Antimicrobial Properties. *J. Med. Chem.* **1996**, *39*, 4471–4477.
- (104) Wood, J. L.; Stoltz, B. M.; Goodman, S. N.; Onwueme, K. Design and Implementation of an Efficient Synthetic Approach to Pyranosylated Indolocarbazoles: Total Synthesis of (+)-RK286c, (+)-MLR-52, (+)-Staurosporine, and (–)-TAN-1030a. *J. Am. Chem. Soc.* **1997**, *119*, 9652–9661.
- (105) Wood, J. L.; Stoltz, B. M.; Onwueme, K.; Goodman, S. N. The Synthesis of Desamido Analogues of Staurosporine, RK-286c, and TAN-1030a. *Tetrahedron Lett.* **1996**, *37*, 7335–7338.
- (106) Wood, L.; Stoltz, B. M.; Goodman, S. N. Total Synthesis of (+)-RK-286c, (+)-MLR-52, (+)-Staurosporine, and (+)-K252a. *J. Am. Chem. Soc.* **1996**, *118*, 10656–10657.
- (107) Ranelli, F. O.; Ricci, R.; Larocca, L. M.; Maggiano, N.; Capelli, A.; Scambia, G.; Benedetti-Panici, P.; Mancuso, S.; Rumi, C.; Piantelli, M. Growth Inhibitory Effect of Quercetin and Presence of Type-II Estrogen-Binding Sites in Human Colon-Cancer Cell Lines and Primary Colorectal Tumors. *Int. J. Cancer* **1992**, *50*, 486–492.
- (108) NCI PDG Clinical Trial Search, November 1997.
- (109) *R&D Drug News* **1998**, Jan 19.
- (110) Senderowicz, A. M.; Headlee, D.; Stinson, S. F.; Lush, R. M.; Kalil, N.; Villalba, L.; Hill, K.; Steinberg, S. M.; Figg, W. D.; Tompkins, A.; Arbuck, S. G.; Sausville, E. A. Phase I Trial of Continuous Infusion Flavopiridol, a Novel Cyclin-Dependent Kinase Inhibitor, in Patients with Refractory Neoplasms. *J. Clin. Oncol.* **1998**, *16*, 2986–2999.
- (111) Mansuri, M. M.; Murthi, K. K.; Pal, K. Preparation of Analogues of Chromones as Inhibitors of Cyclin-Dependent Kinases. PCT Int. Appl. WO 9716447 A1 970509.
- (112) Sedlacek, H. H.; Czech, J.; Naik, R.; Kaur, G.; Worland, P.; Losiewicz, M.; Parker, B.; Carlson, B.; Smith, A. Flavopiridol (L86 8275; NSC 649890), a New Kinase Inhibitor for Tumor Therapy. *Int. J. Oncol.* **1996**, *9*, 1143–1168.
- (113) Carlson, B. A.; Dubay, M. M.; Sausville, E. A.; Brizuela, L.; Worland, P. J. Flavopiridol Induces G1 Arrest with Inhibition of Cyclin-Dependent Kinase CDK2 and CDK4 in Human Breast Carcinoma Cells. *Cancer Res.* **1996**, *56*, 2973–2978.
- (114) Losiewicz, M. D.; Carlson, B. A.; Kaur, G.; Sausville, E. A.; Worland, P. J. Potent Inhibition of CDC2 Kinase Activity by the Flavonoid L86-8275. *Biochem. Biophys. Res. Commun.* **1994**, *201*, 589–595.
- (115) Schwartz, G. K.; Farsi, D.; Greaney, C.; Werner, J.; Kelsen, D. K. The Protein Kinase C (PKC) Inhibitor Flavopiridol (FLAVO) Significantly Enhances the Cytotoxic Effect of Chemotherapy by Promoting Apoptosis in Gastric Cancer Cells. *Prog. Gastric Res.* **1997**, *1*, 627–629.
- (116) Czech, J.; Hoffman, D.; Naik, R.; Sedlacek, H. Antitumoral Activity of Flavone L 86-8275. *Int. J. Oncol.* **1995**, *6*, 31–36.
- (117) Arguello, F.; Alexander, M.; Sterry, J. A.; Tudor, G.; Smith, E. M.; Kalavar, N. T.; Greene, J. F. Jr.; Koss, W.; Morgan, C. D.; Stinson, S. F.; Siford, T. J.; Alvord, G.; Klabansky, R. L.; Sausville, E. A. Flavopiridol Induces Apoptosis of Normal Lymphoid Cells, Causes Immunosuppression, and has Potent Antitumor Activity In Vivo Against Human Leukemia and Lymphoma Xenografts. *Blood* **1998**, *91*, 2482–2490.
- (118) Schwartz, G. K.; Farsi, D.; Greaney, C.; Werner, J.; Kelsen, D. K. The Protein Kinase C (PKC) Inhibitor Flavopiridol (FLAVO) Significantly Enhances the Cytotoxic Effect of Chemotherapy by Promoting Apoptosis in Gastric Cancer Cells. 2nd International Gastric Cancer Congress, Munich, Germany, Apr 27–30, 1997; pp 627–629.
- (119) Bible, K. C.; Kaufmann, S. H. Cytotoxic Synergy Between Flavopiridol (NSC 649890, L86-8275) and Various Antineoplastic Agents: the Importance of Sequence of Administration. *Cancer Res.* **1997**, *57*, 3375–3380.
- (120) de Azevedo, W. F., Jr.; Mueller-Dieckmann, H.; Schulze-Gahmen, U.; Worland, P. J.; Sausville, E.; Kim, S. Structural Basis for Specificity and Potency of a Flavonoid Inhibitor of Human CDK2, a Cell Cycle Kinase. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 2735–2740.
- (121) Kim, S.; Schulze-Gahmen, U.; Brandsen, J.; Filgueira de Azevedo, W., Jr. Structural Basis for Chemical Inhibition of CDK2. *Prog. Cell Cycle Res.* **1996**, *2*, 137–145.
- (122) Kim, K. Preparation of 2-Thia- or 2-Oxa-flavopiridol Analogues for use as Protein Kinase Inhibitors. PCT Int. Appl. WO 9742949.
- (123) Vesely, J.; Havlicek, L.; Strnad, M.; Blow, J. J.; Donella-Deana, A.; Pinna, L.; Letham, D. S.; Kato, J. Y.; Detivaud, L.; Leclerc, S.; Meijer, L. Inhibition of Cyclin-Dependent Kinases by Purine Analogues. *Eur. J. Biochem.* **1994**, *224*, 771–786.
- (124) Glab, N.; Labidi, N. H.; Qin, L. X.; Trehin, C.; Bergounioux, C.; Meijer, L. Olomoucine, an Inhibitor of the CDC2/CDK2 Kinases Activity, Blocks Plant Cells at the G1 to S and G2 to M Cell Cycle Transitions. *FEBS Lett.* **1994**, *353*, 207–211.
- (125) Abraham, R. T.; Acquarone, M.; Andersen, A.; Asensi, A.; Belle, R.; Berger, F.; Bergounioux, C.; Brunn, G.; Buquet-Fagot, C.; Fagot, D.; Glab, N.; Goudeau, M.; Guerrier, P.; Houghton, P. J.; Hendriks, H.; Kloareg, B.; Lippai, M.; Marie, D.; Maro, B.;

- Meijer, L.; Mester, J.; Mulner-Lorillon, O.; Poulet, S. A.; Schierenberg, E.; Schutte, B.; Vaulot, D.; Verhac, M. H. Cellular Effects of Olomoucine, an Inhibitor of Cyclin-Dependent Kinases. *Biol. Cell* 1995, 83, 105–120.
- (126) Ongkeko, W.; Fergusson, J. P.; Harris, A. L.; Norbury, J. Inactivation of CDC2 Increases the Level of Apoptosis Induced by DNA Damage. *J. Cell Sci.* 1995, 108, 2897–2904.
- (127) Schutte, B.; Nieland, L.; van Engeland, M.; Henfling, M.; Meijer, L.; Ramaekers, F. The Effect of the Cyclin-Dependent Kinase Inhibitor Olomoucine on Cell Cycle Kinetics. *Exp. Cell. Res.* 1997, 236, 4–15.
- (128) Deazevedo, W. F.; Leclerc, S.; Meijer, L.; Havlicek, L.; Strand, M.; Kim, S. H. Inhibition of Cyclin-Dependent Kinases by Purine Analogues-Crystal Structure of Human CDK2 Complexed with Roscovitine. *Eur. J. Biochem.* 1997, 243, 518–526.
- (129) Schulze-Gahmen, U.; Brandsen, J.; Jones, H. D.; Morgan, D. O.; Meijer, L.; Vesely, J.; Kim, S. H. Multiple Modes of Ligand Recognition: Crystal Structures of Cyclin-Dependent Protein Kinase 2 in Complex with ATP and Two Inhibitors, Olomoucine and Isopentenyladenine. *Proteins: Struct. Funct. Genet.* 1995, 22, 378–391.
- (130) Hajdich, M. 10th NCI-EORTC Symposium on New Drugs in Cancer Therapy, Amsterdam, The Netherlands, June 16–19, 1998; Abstr. 522.
- (131) Gray, N. S.; Wodicka, L.; Thunnissen, A.-M. W. H.; Norman, T. C.; Kwon, S.; Espinoza, H.; Morgan, D. O.; Barnes, G.; LeClerc, S.; Meijer, L.; Kim, S.-H.; Lockhart, D. J.; Schultz, P. G. Exploiting Chemical Libraries, Structure, and Genomics in the Search for Kinase Inhibitors. *Science* 1998, 281, 533–538.
- (132) Schow, S. R.; Mackman, R. L.; Blum, C. L.; Brooks, E.; Horsma, A. G.; Joly, A.; Kerwar, S. S.; Lee, G.; Shiffman, D.; Nelson, M. G.; Wang, X.; Wick, M. M.; Zhang, X.; Lum, R. T. Synthesis and Activity of 2,6,9-Trisubstituted Purines. *Bioorg. Med. Chem. Lett.* 1997, 7, 2697–2702.
- (133) Brooks, E. E.; Gray, N. S.; Joly, A.; Kerwar, S. S.; Lum, R.; Mackman, R. L.; Norman, T. C.; Rosete, J.; Rowe, M.; Schow, S. R.; Schultz, P. G.; Wang, X.; Wick, M. M.; Shiffman, D. CVT-313, a Specific and Potent Inhibitor of CDK2 that Prevents Neointimal Proliferation. *J. Biol. Chem.* 1997, 272, 29207–29211.
- (134) Lum, R. T.; Blum, C. L.; Mackman, R.; Wick, M. M.; Schow, S. R. Preparation of Purine Inhibitors of Cyclin Dependent Kinase 2 and I $\kappa$ B- $\alpha$  Kinase for use as Antitumor, Antiproliferative, and Leukemia Inhibiting Agents. PCT Int. Appl. WO 9805335 A1 980212.
- (135) Brooks, E. E.; Gray, N. S.; Joly, A.; Kerwar, S. S.; Lum, R.; Mackman, R. L.; Norman, T. C.; Rosete, J.; Rowe, M.; Schow, S. R.; Schultz, P. G.; Wang, X.; Wick, M. M.; Shiffman, D. CVT-313, a Specific and Potent Inhibitor of CDK2 that Prevents Neointimal Proliferation. *J. Biol. Chem.* 1997, 272, 29207–29211.
- (136) Legraverend, M.; Ludwig, O.; Bisagni, E.; Leclerc, S.; Merijer, L. Synthesis of C2 Alkynylated Purines, a New Family of Potent Inhibitors of Cyclin-Dependent Kinases. *Bioorg. Med. Chem. Lett.* 1998, 8, 793–798.
- (137) Kent, L. L.; Hull-Campbell, N. E.; Lau, T.; Wu, J. C.; Thompson, S. A.; Nori, M. Characterization of Novel Inhibitors of Cyclin-Dependent Kinases. *Biochem. Biophys. Res. Commun.* 1999, 260, 768–774.
- (138) Luzzio, M. J.; Bramson, N.; Dickerson, S.; Frye, S. V.; Harris, P.; Hassell, A. M.; Holmes, W.; Hunter, R.; Kuyper, L.; Lackey, K.; Lovejoy, B.; Montana, V.; Rotque, W.; Shewchuk, L.; Veal, J.; Walker, D. Inhibitors of Cyclin Dependent Kinases: Design, Synthesis & Evaluation. *Am. Assoc. Cancer Res.* 1999, Apr 10–14.
- (139) Davis, S. T.; Dickerson, S. H.; Frye, S. V.; Harris, P. A. Substituted Oxindole Derivatives as Protein Tyrosine Kinase and as Protein Serine/Threonine Kinase Inhibitors. PCT Int. Appl. WO9915500 A1 990401.
- (140) Walker, D. H.; Luzzio, M.; Veal, J.; Dold, K.; Edelstein, M.; Parker, P.; Rusnak, D.; Emerson, D.; Miller, C.; Onori, J.; Bramson, H. N.; Harris, P.; Hunter, R.; Dickerson, S.; Kuyper, L. The Novel Cyclin Dependent Kinase Inhibitors, GW5181 and GW9499 Regulate Cell Cycle Progression and Induce Tumor-Selective Cell Death. *Am. Assoc. Cancer Res.* 1999, Apr 10–14.
- (141) Cheung, M.; Glennon, K. C.; Lackey, K. E.; Peel, M. R. Preparation of Arylmethyleneazaxindoles as Protein Kinase Inhibitors. PCT Int. Appl. WO9921859 A1 990506.
- (142) Misra, R. N.; Kimball, S. D.; Rawlins, D. B.; Webster, K. R.; Bursuker, I. Use of Pyrazolo [3,4-b] Pyridine as Cyclin Dependent Kinase Inhibitors. PCT Int. Appl. WO9930710 A1 990624.
- (143) Ruetz, S.; Woods-Conk, K.; Solf, R.; Meyer, T.; Zimmerman, J.; Fabbro, D. Effect of CGP 60474 on Cyclin Dependent Kinases, Cell Cycle progression and onset of Apoptosis in Normal and Transformed Cells. Cold Spring Harbor Winter Conference on Pathways to Cancer, Mar 11–14, 1998; Abstr. 38.
- (144) Furet, P.; Imbach, P.; Capraro, H.; Ruetz, S.; Meyer, T.; Zimmerman, J. Structure-Based Optimization of Olomoucine, a CDK1/2 Inhibitor. 216th ACS National Meeting, Boston, MA, Aug 23–27, 1998.
- (145) Boschelli, D. H.; Dobrusin, E. M.; Doherty, A. M.; Fattacy, A.; Fry, D. W.; Barvian, M. R.; Kallmeyer, S. T.; Wu, Z. Preparation of Pyrido[2,3-d]pyrimidines and 4-Aminopyrimidines as Inhibitors of Cellular Proliferation. PCT Int. Appl. WO 9833798 A2 980806.
- (146) Lundgren, K.; Price, S. M.; Excobar, J.; Huber, A.; O'Connor, P.; Chong, W.; Duvadie, R.; Li, L.; Chu, S. S.; Nonomiyama, J.; Tucker, K.; Mroczkowski, B.; Lewis, C. Novel Cyclin-Dependent Kinase Inhibitors with Potent Growth Inhibitory Action. *Am. Assoc. Cancer Res.* 1999, Apr 10–14.
- (147) Kim, K. S.; Kimball, S. D.; Poss, M. A.; Misra, R. N.; Cai, Z.-W.; Rawlins, D. B.; Webster, K.; Hunt, J. T.; Han, W.-C. Aminothiazole Inhibitors of Cyclin Dependent Kinases. PCT. Int. Appl. WO9924416 A1 990520.
- (148) Kitagawa, M.; Okabe, T.; Ogino, H.; Matsumoto, H.; Suzuki-Takahashi, I.; Kokubo, T.; Higashi, H.; Saitoh, S.; Taya, Y.; Yasuda, H.; Ohba, Y.; Nishimura, S.; Tanaka, N.; Okuyama, A. Butyrolactone I, a Selective Inhibitor of CDK2 and CDC2 Kinase. *Oncogene* 1993, 8, 2425–2432.
- (149) Someya, A.; Tanaka, N.; Okuyama, A. Inhibition of Cell Cycle Oscillation by DNA Replication by a Selective Inhibitor of the CDC2 Kinase Family, Butyrolactone I, in Xenopus Egg Extracts. *Biochem. Biophys. Res. Commun.* 1994, 198, 536–545.
- (150) Nishio, K.; Ishida, T.; Arioka, H.; Kurokawa, H.; Fukuoka, K.; Nomoto, T.; Fukumoto, H.; Yokote, H.; Saijo, N. Antitumor Effects Of Butyrolactone-I, a Selective Cdc2 Kinase Inhibitor, On Human Lung-Cancer Cell-Lines. *Anti-Cancer Res.* 1996, 16, 3387–3395.
- (151) Zaharevitz, D.; Kunick, C.; Schultz, C.; Meijer, L.; Leost, M.; Gussio, R.; Senderowicz, A.; Lahusen, T.; Sausville, E. The Paullones, A Novel Class of Small Molecule Inhibitors of Cyclin-Dependent Kinases. *Am. Assoc. Cancer Res.* 1999, Apr 10–14.
- (152) Okumura, E.; Seki, T.; Hisanaga, S.; Tachibana, K.; Kishimoto, T. Initial Triggering of M Phase in Starfish Oocytes: A Possible Novel Component of Maturation-Promoting Factor Besides CDC2 Kinase. *J. Cell. Biol.* 1996, 132, 125–135.
- (153) Schultz, C.; Link, A.; Leost, M.; Zaharevitz, D. W.; Gussio, R.; Sausville, E. A.; Meijer, L.; Kunick, C. Paullones, a Series of Cyclin-Dependent Kinase Inhibitors: Synthesis, Evaluation of CDK1/Cyclin B Inhibition, and in Vitro Antitumor Activity. *J. Med. Chem.* 1999, 42, 2909–2919.
- (154) Siavoshian, S.; Blottiere, H. M.; Cherbut, C.; Galmiche, J.-P. Butyrate Stimulates Cyclin D and p21 and Inhibits Cyclin-Dependent Kinase 2 Expression in HT-29 Colonic Epithelial Cells. *Biochem. Biophys. Res. Commun.* 1997, 232, 169–172.
- (155) Cui, C.-B.; Ubukata, M.; Kakeya, H.; Onose, R.; Okada, I.; Takahashi, K.; Isono, K.; Osada, H. *J. Antibiot.* 1996, 46, 216–219.
- (156) Ayer, W. A.; Attah-Poku, S. K.; Browne, L. M.; Orszansda, H. *Can. J. Chem.* 1987, 65, 765–769.
- (157) Nomoto, S.; Mori, K. Synthetic microbial chemistry. Part 30. Synthesis of Acetophthalidin, a Fungal Metabolite Which Inhibits the Progression of the Mammalian Cell Cycle. *Liebigs Ann. Recueil.* 1997, 4, 721–723.
- (158) Bojanowski, K.; Nishio, K.; Fukuda, M.; Larsen, A. K.; Saijo, N. Effect of Suramin on p34CDC2 Kinase In Vitro and in Extracts From Human H69 Cells: Evidence for a Double Mechanism of Action. *Biochem. Biophys. Res. Commun.* 1994, 203, 1574–1580.
- (159) Ip, C.; Hayes, C.; Budnick, R. M.; Ganther, H. E. Chemical Form of Selenium, Critical Metabolites and Cancer Prevention. *Cancer Res.* 1991, 51, 595–600.
- (160) Ip, C.; Ganther, H. E. Relationship Between the Chemical Form of Selenium and Anticarcinogenic Activity. In *Cancer Chemoprevention*; Wattenberg, L.; Lipkin, M.; Boone, C. W., Kelloff, G. J., Eds.; CRC Press: Boca Raton, FL, 1992; pp 479–488.
- (161) Sinha, R.; Medina, D. Inhibition of CDK2 Kinase Activity by Methylselenocysteine in Synchronized Mouse Mammary Epithelial Tumor Cells. *Carcinogenesis* 1997, 18, 1541–1547.
- (162) Fahraeus, R.; Lane, D. P. INK4 Protein p16-Derived Peptides or Peptide Mimetics that Bind by Cyclin-Dependent Kinases, Inhibit Rb Protein Phosphorylation, and are Useful for Treating Hyperproliferative Disorders. PCT Int. Appl. WO 9711174 A1 970327.
- (163) Sasaki, S.; Hashimoto, T.; Obana, N.; Yasuda, H.; Uehara, Y.; Maeda, M. Design of New Inhibitors for CDC2 Kinase Based on a Multiple Pseudosubstrate Structure. *Bioorg. Med. Chem. Lett.* 1998, 8, 1019–1022.
- (164) Buckley, M. F.; Sweeney, K. J.; Hamilton, J. A.; Sini, R. L.; Manning, D. L.; Nicholson, R. I.; deFazio, A.; Watts, C. K.; Musgrove, E. A.; Sutherland, R. L. Expression and Amplification of Cyclin Genes in Human Breast Cancer. *Oncogene* 1993, 8, 2127–2133.

- (165) Gillett, C.; Fantl, V.; Smith, R.; Fisher, C.; Bartek, J.; Dickson, C.; Barnes, D.; Peters, G. Amplification and Overexpression of Cyclin D1 in Breast Cancer Detected by Immunohistochemical Staining. *Cancer Res.* **1994**, *54*, 1812–1817.
- (166) Bartkova, J.; Lukas, J.; Strauss, M.; Bartek, J. Cyclin D1 Oncoprotein Aberrantly Accumulates in Malignancies of Diverse Histogenesis. *Oncogene* **1995**, *10*, 775–778.
- (167) Wolfel, T.; Hauer, M.; Schneider, J.; Serrano, M.; Wolfel, C.; Klehmann-Hieb, E.; De Plaen, E.; Hankeln, T.; Meyer zum Buschenfelde, K. H.; Beach, D. A p16INK4a-Insensitive CDK4 Mutant Targeted by Cytolytic Lymphocytes in a Human Melanoma. *Science* **1995**, *269*, 1281–1284.
- (168) Wunder, J. S.; Eppert, K.; Burrow, S. R.; Gogkz, N.; Bell, R. S.; Andrulis, I. L. Co-amplification and Overexpression of CDK4, SAS and MDM2 Occurs Frequently in Human Parosteal Osteosarcomas. *Oncogene* **1999**, *18*, 783–788.
- (169) Kamb, A. Role of a Cell Cycle Regulator in Hereditary and Sporadic Cancer. *Cold Spring Harb. Symp. Quantum Biol.* **1994**, *59*, 39–47.
- (170) Liggett, W. H., Jr.; Sidransky, D. Role of the p16 Tumor Suppressor Gene in Cancer. *J. Clin. Oncol.* **1998**, *16*, 1197–1206.
- (171) Sherr, C. J.; Roberts, J. M. CDK Inhibitors: Positive and Negative Regulators of G1-Phase Progression. *Genes Dev.* **1999**, *13*, 1501–1512.
- (172) Rane, S. G.; Dubus, P.; Mettus, R. V.; Galbreath, E. J.; Boden, G.; Reddy, E. P.; Barbacid, M. Loss of Cdk4 Expression Causes Insulin-deficient Diabetes and Cdk4 Activation Results in Beta-islet Hyperplasia. *Nat. Genet.* **1999**, *22*, 44–52.
- (173) Tsutsui, T.; Hesabi, B.; Moons, D. S.; Pandolfi, P. P.; Hansel, K. S.; Koff, A.; Kiyokawa, H. Targeted Disruption of CDK4 Delays Cell Cycle Entry with Enhanced p27<sup>Kip1</sup> Activity. *Mol. Cell. Biol.* **1999**, *19*, 7011–7019.
- (174) Van den Heuvel, S.; Harlow, E. Distinct Roles for Cyclin-dependent Kinases in Cell Cycle Control. *Science* **1993**, *262*, 2050–2054.
- (175) Cheng, M.; Olivier, P.; Diehl, J. A.; Fero, M.; Roussel, M. F.; Roberts, J. M.; Sherr, C. J. The p21(Cip1) and p27(Kip1) CDK Inhibitors are Essential Activators of Cyclin D-dependent Kinases in Murine Fibroblasts. *EMBO J.* **1999**, *18*, 1571–1583.
- (176) McConnell, B. B.; Gregory, F. J.; Stott, F. J.; Hara, E.; Peters, G. Induced Expression of p16ink41 Inhibits Both CDK4 and CDK2 Associated Kinase Activity by Reassortment of Cyclin-CDK Inhibitor Complexes. *Mol. Cell. Biol.* **1999**, *19*, 1981–1989.
- (177) Quelle, D. E.; Zindy, F.; Ashmun, R. E.; Sherr, C. J. Alternate Reading Frames of the INK4a Tumor Suppressor Gene Encode Two Unrelated Proteins Capable of Inducing Cell Cycle Arrest. *Cell* **1995**, *83*, 993–1000.
- (178) Sharpless, N. E.; DePinho, R. A. The INK4A/ARF Locus and its Two Gene Products. *Curr. Opin. Genet. Dev.* **1999**, *9*, 22–30.
- (179) Larsen, C. J. Contribution of the Dual Coding Capacity of the p16INK4a/MTS1/CDKN2 Locus to Human Malignancies. *Prog. Cell Cycle Res.* **1997**, *3*, 109–124.
- (180) Zhang, Y.; Xiong, Y.; Yarbrough, W. G. ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a Locus Deletion Impairs Both the Rb and p53 Tumor Suppressor Pathways. *Cell* **1998**, *92*, 725–734.
- (181) Pomerantz, J.; Schreiber-Agus, N.; Liegeois, N. J.; Silverman, A.; Alland, L.; Chin, L.; Potes, J.; Chen, K.; Orlow, I.; Lee, H. W.; Cordon-Cardo, C.; DePinho, R. A. The Ink4a Tumor Suppressor Gene Product, p19Arf, Interacts with MDM2 and Neutralizes MDM2's Inhibition of p53. *Cell* **1998**, *92*, 713–723.
- (182) Stott, F. J.; Bates, S.; James, M. C.; McConnell, B. B.; Starborg, M.; Brookes, S.; Palmero, I.; Ryan, K.; Hara, E.; Vousden, K. H.; Peters, G. The Alternate Product from the Human CDKN2A Locus, p14(ARF), Participates in a Regulatory Feedback Loop with p53 and MDM2. *EMBO J.* **1998**, *17*, 5001–5014.
- (183) Honda, R.; Yasuda, H. Association of p19(ARF) with Mdm2 Inhibits Ubiquitin Ligase Activity of Mdm2 for Tumor Suppressor p53. *EMBO J.* **1999**, *18*, 22–27.
- (184) Tao, W.; Levine, A. J. P19(ARF) stabilizes p53 by blocking nucleo-cytoplasmic shuttling of Mdm2. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6937–6941.
- (185) Murphy, M.; Stinnakre, M. G.; Senamaud-Beaufort, C.; Winston, N. J.; Sweeney, C.; Kubelka, M.; Carrington, M.; Brechot, C.; Sobczak-Thépot, J. Delayed Early Embryonic Lethality Following Disruption of the Murine Cyclin A2 Gene. *Nat. Genet.* **1997**, *15*, 83–86.
- (186) Fang, F.; Orend, G.; Watanabe, N.; Hunter, T.; Ruoslahti, E. Dependence of Cyclin E-CDK2 Kinase Activity on Cell Anchorage. *Science* **1996**, *271*, 499–502.
- (187) Orend, G.; Hunter, T.; Ruoslahti, E. Cytoplasmic Displacement of Cyclin E-cdk2 Inhibitors p21Cip1 and p27Kip1 in Anchorage Independent Cells. *Oncogene* **1998**, *16*, 2575–2583.
- (188) Chen, Y. N.; Sharma, S. K.; Ramsey, T. M.; Jiang, L.; Martin, M. S.; Baker, K.; Adams, P. D.; Bair, K. W.; Kaelin, W. G., Jr. Selective Killing of Transformed Cells by Cyclin/Cyclin-dependent Kinase 2 Antagonists. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4221–4223.
- (189) Hunter, T. 1001 Protein Kinases Redux - Towards 2000. *Cell* **1994**, *5*, 367–376.

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